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

Editor in Chief: C. Saiz-Jimenez
Assistant Editor: M.A. Rogerio

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ICBM

Newsletter No. 4
March 2002

Special Issue: COALITION Workshop "Databases of living Microorganisms from Stone Monuments", Gent, Belgium, March 9 2001

Index

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BACTERIAL DETERIORATION OF MONUMENTS: DISCUSSION OF SOME RESEARCH PROBLEMS AND THE CONSTRUCTION OF A DATABASE OF WELL CHARACTERISED STRAINS

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It is well recognised that bacteria can be involved in the deterioration of monuments (Ciferri 1999, Warscheid and Braams 2000). Although biodeterioration phenomena were given more attention in more recent research, several problems remain. The objective of this report is to sum-up some of the problems of the research on bacteria associated with biodeterioration of monuments and to explain in what way a database as conceived in COALITION could help to deal with some of these problems.

Sum-up of some research problems

A first problem is related to the characterisation of cultured strains. Many bacteria related with biodeterioration lack a reliable characterisation at the species level (e.g. discussed in Heyrman and Swings 2001). If different researchers want to compare their results good characterisations are a prerequisite. Otherwise it cannot be stated that the same bacterial species were found on different objects of art. In the light of future conservation it is very important to make such comparisons, since only in this way general conclusions can be drawn from the different researches. Thus, several new species associated with biodeterioration should be described. On the other hand the results can also be compared more easily if techniques that are used are linked to an accessible and a very extensive database. 16S rDNA sequencing is an example of such a technique.

A second problem is formed by the uncultured part of the bacterial flora. The use of DGGE analysis in this field of research clearly revealed this problem, a large part of the community can only be detected but cannot be studied in laboratory experiments. Of course, from the moment the genera are known, special media can be applied to culture the bacteria, but this is not successful in all cases. An additional problem is the large discrepancy between the cultivation results and those obtained by DGGE, it was shown that both techniques can result in a totally different bacterial diversity for the same sample (e.g. Gurtner et al. 2000). For the moment it can be stated that a combined approach raises the change of characterising the total bacterial community.

A third problem is encountered in the study of the deteriorative potential of the bacteria and originates from the fact that the identification of the causative agents can only be achieved when pure cultures isolated from the damaged substrate are individually tested in laboratory tests. The effect of the combined flora and the situation in situ can thus not be estimated. Also the role of the unculturables is difficult to study.

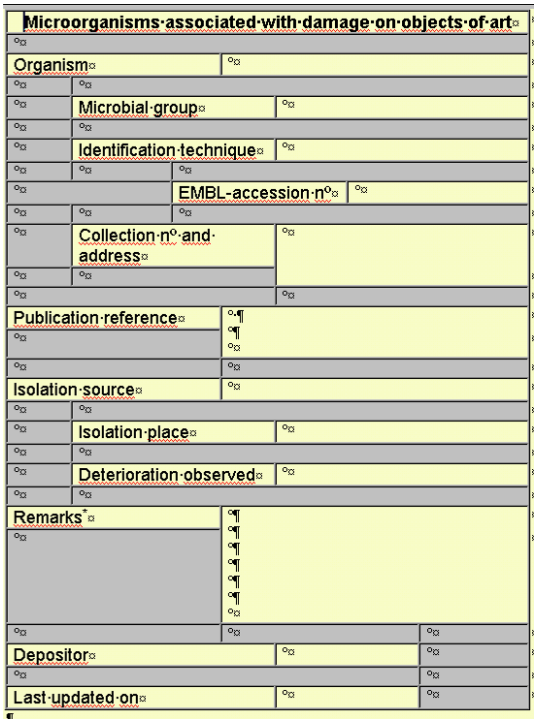
Usefulness of a database of well characterised strains

The purpose of the construction of a database of living microorganisms from stone monuments is to deal with some of the cited problems. The database is conceived as a list of microorganisms available on the world wide web, that are well characterised and are present in a collection that guarantees its maintenance, purity and distribution. In the field of characterisation a set of bacteria present in the database can be withdrew from the cited collections and can then be included in the characterisation used for new bacterial isolates. In this way a laboratory database of a certain technique can be expanded with profiles of well-studied bacteria, isolated from other monuments. In the study of the deteriorative potential of bacterial isolates the database can provide well characterised strains from different monuments that can be included in the laboratory experiments. On the other hand new isolates can be compared with strains of which the biodeteriorative potential is well known. The biggest problem is the unculturables.

DGGE analysis results in a collection of sequences that are submitted to the EMBL database. New sequences resulting from DGGE can easily be compared with those present in this database. Also cultured bacteria can be compared, but only through sequence analysis.

Practical use of the database

The database is still under construction, but will become publically accesible in the course of 2002 on the website of COALITION. The figure below shows the data form that should be filled in to enter information on a strain that meets the database requirements: well characterised and maintained in a collection that guarantees its maintance, purity and distribution.



Different information fields were used, which should provide sufficient information for comparison or to choose a strain for a specific experiment, although the information is limited to ensure an easy registration of the strains. The database on the world wide web will give a list of the different strains that were entered, sorted by species name. The biggest difference with other lists of bacteria (e.g. collection catalogues), is that for each strain the type of monument and the deterioration observed is listed. On this basis a researcher can choose an interesting strain and apply for it at the respective collection.

One example of strains that will be included in the database are the micro-organisms isolated and characterised in the EU-project MICROCORE (ENV4-CT98-0705), a study on the microbial community of three European mural paintings showing different deterioration phenomena. In regard to bacteria, approximately 50 strains, all studied by 16S rDNA sequence analysis and conserved in the BBCM/LMG Culture Collection (Gent, Belgium), will be put in the database. Many of these mural painting strains could not be attributed to any known species. Therefore, several of the isolates are now in the process of description, e.g. *Halomonas muralis*, *Brachybacterium fresconis* and *B. sacelli* (submitted for publication), and will be added to the database after acceptation.

References

Ciferri, O. (1999). Microbial degradation of paintings. Appl. Environ. Microbiol. 65, 879-885.

Gurtner, C., Heyrman, J., Piñar, G., Lubitz, W., Swings, J., Rölleke, S. (2000). Comparative analyses of the bacterial diversity on two different biodeteriorated wall paintings by DGGE and 16S rDNA sequence analysis. Int. Biodet. Biodeg. 46, 229-239.

Heyrman, J. and Swings, J. (2001). 16S rDNA sequence analysis of bacterial isolates from biodeteriorated mural paintings in the Servilia tomb (Necropolis of Carmona, Seville, Spain). Syst. Appl. Microbiol. 24, 417-422.

Warscheid, T. and Braams, J. (2000). Biodeterioration of stone: a review. Int. Biodet. Biodeg. 46, 343-368.

FUNGI ON STONE MONUMENTS: STATE OF THE ART

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Alteration phenomena caused by fungi

Materials can be altered and destroyed by fungi in numerous ways that were intensively discussed in the literature and will only be summed up here:

- Fungal production of organic acids leads to dissolution of carbonate rock and to decohesion of calcite-bound quartz grains in sandstone. Also CO_2 released by the fungi can act as an

acid (carbonic acid) if it is dissolved in water.
- Color change by fungal pigments (mostly melanin and carotenoids).
- Degradation of organic compounds (e.g. skin glue, egg) in paintings.
- Change in porosity of materials.
- Decohesion of rock grains by internal pressure causes serious material losses as e.g. pitting of marble.

Fungal diversity on rock monuments

The fungal diversity on rock monuments is determined by three main factors:

(a) The physical and chemical properties of the rock. Porous rock material is usually inhabited by a higher number of fungi because living conditions inside the rock are more moderate than on the surface. More water is retained inside the rock, UV-radiation is much weaker and the amplitude of temperature and humidity changes is lower.

(b) The climatic conditions: with growing temperature, decreasing humidity and increasing UV-radiation the diversity of fungi on monuments is decreasing because extreme conditions necessitate a higher specialization and adaptation of the organisms.

(c) The availability of organic nutrients: organic nutrients can either be provided by the accompanying autotrophic organisms on the materials as e.g. cyanobacteria and algae or originate from the air (dust, dirt particles, carbohydrates, aromatic compounds).

The rock fungal community can be divided into two major groups:

(a) Hyphomycetes and Coelomycetes like *Alternaria*, *Fusarium*, *Penicillium*, *Trichoderma*, *Phoma* and many others that are commonly known as typical soil fungi. Most members of these genera have in common that they need a relatively high water potential, eutrophic nutrient conditions and moderate growth temperatures.

(b) Meristematic fungi and black yeasts are to be found on monuments, in the phyllosphere and as opportunists and pathogenes of mammals. They have in common that they are highly stress resistant and endure long periods of desiccation, low nutrient support, high temperatures and UV-radiation. The stress resistance is due to several adaptations like thick melanized cell walls, a special morphology with an optimal volume surface ratio and the production of trehalose as enzyme stabilizing agent.

On most monuments both groups of fungi can be found but the ratio changes with the climatic region (Fig. 1). In humid areas as in North Germany the fungal community is dominated by the hyphomycetes, in Vienna with relatively dry and warm summers the ratio of meristematic fungi and black yeasts is increased and in the Mediterranean area only few hyphomycetes occur whereas a lot of different species of meristematic fungi and black yeasts inhabit the monuments.

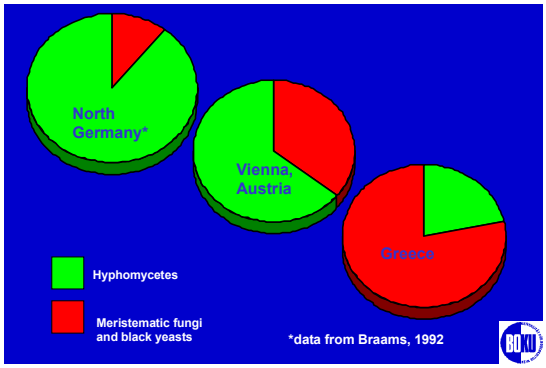

Fig. 1. The ratio of hyphomycetes versus meristenmatic fungi and black yeasts in different climatic regions.

Molecular data on rock fungi to be collected in a databank

Due to the fact that a lot of fungi either show a very variable morphology that changes with environmental conditions or lack discriminative features like the meristematic fungi, mycologists today generally agree that the identification of fungi is only reliable based on molecular data. Thus, also for monument fungi databanks containing molecular data are urgently needed in order to be able get satisfactory identification results and to compare results from different locations and carried out by different scientist. Of course, data in such a databank must be

compatible with large databanks like EMBL.

The most important data that should be collected are:

(a) The complete 18S rDNA sequence of the fungi allows the identification of the families and genera in most cases.
(b) The ITS1 and ITS2 sequencing data are more variable and thus useful for species identification in a lot of genera.
(c) The sequencing of conserved structural protein-encoding genes like β-tubuline is necessary for some genera of fungi (as e.g. *Fusarium*) because the species cannot be distinguished on the ITS-sequences.
(d) Restriction maps of the fungi can be very useful for the rapid identification and comparison of strains by genetic fingerprints.

Future approaches for the use of databank information

Sequence data stored in databanks are the basis for very important ecological studies. The SSU and LSU sequencing data are necessary for the design of genus and species specific primers for the in-situ detection via hybridization and for the analysis of fungal communities via Denaturing Gradient Gel Electrophoresis or Single Strand Conformation Polymorphism. Restriction patterns are necessary for the characterization of fungal communities with T-RFLP. The β-tubuline genes turned out to be suited for the species specific PCR-detection.

For a review on "Fungi as geologic agents" see Sterflinger (2000). Geomicrobiol. J. 17, 97-124.

DATA BANKS ON CYANOBACTERIA AND ALGAE

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Information about data banks on cyanobacteria and algae can be found in the WWW. In fact, there are particular web sites such as GenBank, or Cyanosite which give free information about gene sequences, collections, media and references about cyanobacteria and algae. Moreover a lot of data banks are reserved to different research projects and the consultation of these are allowed only to the staff of the projects.

In this report some of the most important web sites referred to databanks on cyanobacteria and algae are mentioned.

Cell/GenBanks NCBI
http://www.ncbi.nlm.nih.gov/Genbank/GenbankOverview.html

The National Centre for Biotechnology Information (NCBI) posses different data banks linked and integrated each other.

These databases include nucleotide sequences, protein sequences, macromolecular structures, whole genomes, and MEDLINE, through PubMed. They have number of information also for cyanobacteria and algae. *Entrez* is a search and retrieval system that integrates information from databases at NCBI.

GenBank is the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences.

There are approximately 15,850,000,000 bases in 14,976,000 sequences records as of December 2001. A new release is made every two months. GenBank is part of the International Nucleotide Sequence Database Collaboration, which is comprised of the

1.- DNA DataBank of Japan (DDBJ),
2.- the European Molecular Biology Laboratory (EMBL), and
3.- GenBank at NCBI.

These three organizations exchange data on a daily basis.

Each GenBank entry includes a concise description of the sequence, the scientific name and taxonomy of the source organism, and a table of features

that identifies coding regions and other sites of biological significance, such as transcription units, sites of mutations or modifications, and repeats.

Most sequence analysis programs on PSC supercomputers are capable of reading in GenBank data in the GenBank flat file format.

The collaboration that exists among the International Nucleotide Sequence Databases has led to many beneficial projects that promise to proliferate in the molecular biology community.

Currently, the following projects are part of the collaborative effort among the three databases:

The Taxonomy Project

One of the goals of the collaborators is to use a unified taxonomy across all databases, largely one based on sequence information.

The taxonomy project was set up as a tool for biologists world-wide, and also as a shared instrument for the collaborators. This is one of the important resources used for the maintenance of Genetic Codes, important for the correct translation of coding sequences.

The Feature Table

The Feature Table documentation represents the shared rules that allow the three databases to exchange data on a daily basis. The Feature Table represent the vocabulary that is used to describe the DNA sequence annotations as well as that of the protein sequence(s) they encode.

Access to GenBank

GenBank is available for searching at NCBI via several methods.

The GenBank database is designed to provide and encourage access within the scientific community to the most up to date and comprehensive DNA sequence information. Therefore, NCBI places no restrictions on the use or distribution of the GenBank data.

Submissions to GenBank

The most important source of new data for GenBank is direct submissions from scientists. NCBI provides timely and accurate processing and biological review of new entries and updates to existing entries, and is ready to assist authors who have new data to submit and keeps the database as comprehensive, current, and accurate as possible. Essentially, there are two principal ways, BankIt and Sequin. BankIt is a Web submission tool and recommended for simple submissions.

- **BankIt** allows you to enter sequence information into a form, edit as necessary, and add biological annotation (e.g., coding regions, mRNA features). BankIt transforms your data into GenBank format for your review and when your record is completed it can be submitted directly to GenBank. You have the option of adding information by using text boxes to describe in your own words the source of the sequence and its biological features. The GenBank annotation staff reviews the submitted textual information, incorporates it into the appropriate structured fields, and returns the record by e-mail for your review. When using BankIt, the prepared sequence entries are submitted directly to GenBank through the WWW.

- **Sequin** is an interactive, graphically-oriented program based on screen forms and controlled vocabularies that guides you through the process of entering your sequence and providing biological and bibliographic annotation. Sequin is designed to simplify the sequence submission process and to provide graphical viewing and editing options. It incorporates robust error checking and accommodates very long sequences and complex annotations.

Searching the database with a query sequence

There are number of programs that can be used to search the GenBank database with a query sequence.

Retrieving a database entry
- On the Sequence Analysis Resource through the GCG Wisconsin software.

- On the VMS front ends through the GCG Wisconsin software.

Genetics Computer Group (GCG) Wisconsin Package is a program suite for nucleic acid and protein sequence analysis. GCC programs manipulate, analyse, and display nucleic acid and protein sequences. GCG complements the more computationally intensive analysis programs available at the PSC.

Pairwise Database Searching and Alignment software
FSCHIFT, compares a protein query sequence with a translated DNA library sequence.

MAXSEGS, an optimal local sequence alignment program that will find the N-best alignments between two nucleic acid sequences or two protein sequences.

NWGAP, a global sequence alignment program.

ST, fast database searching program that compares a protein sequence against the sequences in a protein data bank.

It is also possible to obtain a cyanobacterial sequence from GenBank choosing the microorganism and the gene sequence. The result shows information about the organism, the gene, the authors, publications and so on.

CyanoBase
http://www.kazusa.or.jp/cyano/index.html

CyanoBase provides an easy way of accessing the sequence and all-inclusive annotation data through image maps, keyword searches and the gene category list. This database was originally developed by Makoto Hirosawa, Takakazu Kaneko and Satoshi Tabata. The current version of CyanoBase has been developed and maintained by Yasukazu Nakamura, Takakazu Kaneko, Satoshi Tabata and Nobuyuki Miyajima at Kazusa DNA Research Institute.

The strain chosen for the genome analysis was the unicellular *Synechocystis* sp. strain PCC 6803, with the advantage of its transformable characteristics.

PLMItRNA
http://bighost.area.ba.cnr.it/PLMItRNA

It constitutes a database for tRNA molecules and genes identified in the mitochondria of all green plants. In the database 8 green algae are present.
The current version of PLMItRNA has been realized to constitute a database for tRNA molecules and genes identified in the mitochondria of all green plants (Viridiplantae). The database now contains 436 genes and 16 tRNA entries relative to 25 higher plants (one bryophyta and 24 vascular plants) and, four red algae, eight green algae.

The database has been described in the article:
Damiano F., Gallerani R., Liuni S., Licciulli F. and Ceci L.R. (2001). PLMItRNA, a database for mitochondrial tRNA genes and tRNAs in photosynthetic eukaryotes. Nucleic Acids Res. 29, 167-168.

Cyanosite
http://www-cyanosite.bio.purdue.edu

Cyanosite (on-line in August of 1995), one of the original goals of Cyanosite was to provide materials that may be used in teaching students about cyanobacteria. Toward that goal, an image gallery was started in October of 1997. New links are added regularly and all the links on the site are checked biannually. Nearly 60 media recipes were added to the sites in April 1998.

Announcements for meetings, courses, books, and jobs have been placed on Cyanosite and the webspinner encourages others to submit materials for posting. The webspinner's philosophy has always been to keep it simple and include everybody. A review of Cyanosite was published in the journal Science on 4/24/98 and another review was

published in Genetic Engineering News on 9/15/98.

Culture Collection

ATCC American Type Culture Collection
http://www.atcc.org

Brasilian Microbial Culture Collections
http://www.bdt.org.br/colecoes/microrganismo/colecao

Chinese Culture Collections
http://www.im.ac.cn/en/index.html

CCAP, Culture Centre of Algae and Protozoa, Cambridge (UK)
http://www.ife.ac.uk/ccap

The collection functions as the national service collection of algae and protozoa in the UK and is linked with other service collections world-wide via the World Federation of Culture Collections (WFCC). The CCAP currently maintains approximately 2000 strains of algae and protozoa. Strains are primarily maintained by serial sub-culture although 30% of the algal strains and 2% of the protozoan strains are cryopreserved. The main function of the Collection is to maintain and supply selected cultures of free-living algae and protozoa, together with their associated information, to the research community and industry.

MSDN, Microbial Strain Data Network
http://panizzi.shef.ac.uk/msdn

PCC, Pasteur Culture Collection of Cyanobacteria
http://www.pasteur.fr/recherche/banques/PCC

Culture Collection of axenic cyanobacterial strains officially established in 1976 by the relevant French Ministerial authorities. In compliance with the instructions of the ECCO (European Culture Collections' Organization) the PCC ensures the following services:

- The maintenance of axenic cyanobacterial strains in accordance with established criteria of quality;

- Supply of cyanobacterial strains without restrictions of access other than those imposed by legal and ethical requirements;

- Continuous enlargement of the collection by addition of new strains;

- Publication of a catalogue listing the available strains;

- Characterisation (with prior agreement) of cyanobacterial strains upon the request of qualified public or private groups;

- Free flow of information concerning the cultures (by phone, fax or mail)

All the strains have an identification number and they are divided in 5 sections containing different genera and it is possible to retrieve information about a particular strain such as identification source, medium, etc.

SAG, Culture Collection of Algae at Goettingen University
http://www.gwdg.de/~epsag/phykologia/sag/beginn.htm

U.C., Berkeley's and Jepson Herbaria, University of California at Berkeley
http://ucjeps.herb.berkeley.edu

UTCC, University of Toronto Culture Collection of Algae and Cyanobacteria
http://www.botany.utoronto.ca/utcc

As Canada's national service collection of freshwater algae and cyanobacteria the collection provides research quality cultures and related services to educational institutions, government and commercial laboratories. It is housed in the University of Toronto in the Department of Botany, which also provides support services.

Cultures are listed alphabetically by genus and species and the corresponding information for each isolate. Each strain is assigned a unique number, which will never change.

UTEX, Culture Collection, a searchable source for the cultures at University of Texas
http://www.bio.utexas.edu/research/utex

WDCM, World Data Centre for Microorganisms
http://wdcm.nig.ac.jp

The World Federation for Culture Collections (WFCC) pioneered the development of an international database on culture resources world-wide. The result is the WFCC World Data Center for Microorganisms (WDCM). Searchable index based at RIKEN (Institute of Physical and Chemical Research), Saitama, Japan. This data resource is now maintained at National Institute of Genetics (NIG), Japan and has records of nearly 500 culture collections from 60 countries. The records contain data on the organisation, management, services and scientific interests of the collections.

Each of these records is linked to a second record containing the list of species held. The WDCM database forms an important information resource for all microbiological activity and also acts as a focus for data activities among WFCC members. Its aim is to promote and support the establishment of culture collections and related services, to provide liaison and set up an information network between the collections and their users, to organise workshop and conferences, publication and newsletters and work to ensure the long-term perpetuation of important collections.

DATABASE OF LICHENS: SOURCES OF INFORMATION FOR RESTORERS AND MUSEUM WORKERS

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Introduction

A lichen is a symbiotic association between an alga or cyanobacterium (photobiont) and a fungus (mycobiont), which results in an stable thallus, morphological and physiologically well characterised. The photobiont is in charge of providing carbohydrates, while the mycobiont provides shelter for it. This represents an ecological advantage for the lichen, as it allows the thalli to colonise and grow on a wide range of substrates, even in harsh conditions, in extreme environments.

Lichens are found from the Arctic tundra to the arid desert regions, where flowering plants are absent. Compared with them, lichens can tolerate complete drying out for long periods, and are able to quickly reabsorb water, even from damp air, to become physiologically active again. Lichens can colonise different kind of substrates, from soil, trees and rocks to man-made substrates. We can see them on walls and buildings, as they will colonise stones wherever they find good conditions, and can be really abundant, especially in clean air areas. For instance, in lowland Britain, churchyards play the role of rocks as substrate for lichens, as natural outcrops are absent, and some lichen species are confined exclusively to walls of ancient churches.

Lichens on stones are not necessary harmful. On the contrary, they may acts as a protective layer shielding the stone from atmospheric conditions, which can cause decay, such as wind, rainwater or acid rain. Presence of lichens can indicate that the area has a good air quality, and probably some of the species present can be of interest. As we said before, they are maybe not found elsewhere, and, thus, contribute to the diversity of organisms of the country.

Nevertheless, occasionally, presence of lichens on monuments or buildings may not be desirable, as well for stone decay, as for aesthetics. In these cases, and before deciding the method to be applied for cleaning, it would be good to know which lichens are going to be removed. The idea is: “to know before to act”. Evaluation of biological damage is possible from the knowledge of the lichen species present on the stone.

General information

Checklist of lichens of the European countries

Feuerer, T. Checklists of lichens and lichenicolous fungi. Version 1 January 2002. The global checklist of lichens and lichenicolous fungi will be available in autumn 2002. http://www.biologie.uni-hamburg.de/checklists/europe.htm

Biodiversity of Mediterranean lichens

Currently, lichen checklists of 7 countries are included in this information system: Morocco, Israel, Italy, Slovenia, Tunisia, Turkey and Ukraine. Some of these can be retrieved directly as plain text. Information on 5 countries can be retrieved via the continuously updated on-line database. A forum to enter and discuss new information is available. http://biobase.kfunigraz.ac.at/medlichens.html

Local information

Austria. The complete checklist, including a bibliography, by Türk and Poelt (1993) is not available online. There is a red list by Türk and Hafellner (1999) available online:

Türk, R. and Hafellner, J. (1999) [first posted May 4th, 1999]. Red List of Endangered Lichens in Austria. Internet version. Graz: Karl-Franzens-University, Institute of Botany. http://www-ang.kfunigraz.ac.at/~hafell/redlist.htm

Belgium and Luxembourg. Diederich, P. and Sérusiaux. E. (Coll. Boom, P. P. G. van den and Brand, A.M.). (2000). The lichens and lichenicolous fungi of Belgium and Luxembourg. An annotated checklist. Musée nat. hist. nat., Luxembourg, 207 pp. It is not available online.

Denmark. Checklist of the Danish Lichens. This list is available in print by Ulrik Søchting and Vagn Alstrup from Nordisk Lichenologisk Forening 1989. http://www.bot.ku.dk/groups/mycology/dklaver/vis.asp

Estonia. An updated internet version based on the publication Randlane, T. and A. Saag (Eds), (1999). Second checklist of lichenized, lichenicolous and allied fungi of Estonia. Folia Cryptog. Estonica 35: 1 - 132. It contains 802 lichens, 39 lichenicolous fungi and 22 non-lichenized allied taxa. http://www.ut.ee/lichens

Germany. The total checklist is based on Wirth (1994). It is supplemented by checklists of the 16 states ("Bundesländer"). http://www.biologie.uni-hamburg.de/checklists/germany_l0.htm

Hauck M. (1996). Die Flechten Niedersachsens. Bestand, Ökologie, Gefährdung und Naturschutz. Naturschutz und Landschaftspflege in Niedersachsen 36: 1-208. This checklist is an abridged and updated online version of the printed lichen flora of Lower Saxony (Niedersachsen) by Hauck (1996). http://www.gwdg.de/~mhauck/chklst.htm

Deutschlands. Scholz, P. (2000). Katalog der Flechten und flechtenbewohnenden Pilze Deutschlands. Schriftenreihe für Vegetationskunde, Heft 31: 298 pp. It is not available online.

Iberian Peninsula. Llimona, X. and Hladun, N. (2001). Checklist of the Lichens and lichenicolous Fungi of the Iberian Peninsula and Balearic Islands. Bocconea 14:1-581. It is not available online.

British Isles. The List of 2157 species, updated on 20th September 2001, includes lichenicolous fungi as well as non-lichenized fungi and incorporates all the additions, corrections and deletions since the original List was published on 22nd. March 1999. These changes are shown separately at the end of the main List. Updates are also published in B.L.S. Bulletins. http://www.argonet.co.uk/users/jmgray/numlst.htm

United Kingdom

Hawksworth, D., (2001). Checklist of lichens and lichenicolous fungi of the United Kingdom. Version 1 October 2001. http://www.biologie.uni-hamburg.de/checklists/unitedkingdom_l.htm

Italy. ITALIC is the new information system on Italian lichens searchable on-line since October 2000. It organizes several databases and archives dealing with the lichens of Italy. It originates from the tranformation of the checklist of Italian lichens: Nimis, P.L. (1993). The Lichens of Italy. An annotated catalogue. Museo Regionale di Scienze Naturali, Torino. Monografie 12, 897 pp., into a database published in the internet (Nimis 1999). http://dbiodbs.univ.triestre.it

Norway. Checklist to Norwegian lichens. This checklist is based on Santesson, R. (1993). The lichens and lichenicolous fungi of Sweden and Norway, SBT-förlaget, Lund. Updates are made regularily by Einar Timdal and Tor Tønsberg, the latest on 06.01.1997. http://www.toyen.uio.no/botanisk/lav/LISTS.htm

Slovakia. Checklist of non-vascular and vascular plants of Slovakia. Lichen-Forming Fungi (Lichens) by Ivan Pišút. http://nic.savba.sk/sav/inst/botu/page/checklist.html

The Netherlands. Aptroot, A.; Van Herk, C.M.; Sparrius, L.B. and Boom, P.P.G. van den. (1999). Checklist van de Nederlandse Korstmossen en Lichenicole Fungi, Buxbaumiella 50(1): 4-64. http://www.lichens.myweb.nl/dutch_lichens.htm

This checklist can be downloaded either as HTML document or as an Access database. The HTML-version includes data on Red Listed species.

Identification Keys and Descriptions

Keys are constructed of pairs of contrasting characters. The identification is confirmed or rejected by comparison with photographs and the written description.

Clauzade, G. and Roux, C. (1985). Likenoj de Okcidenta Europo Ilustrita determinlibro. Bulletin de la Société Botanique du Centre-Ouest, n.s., numero spécial 7: 1—893. There are two supplements: 'Supplemento 2a', op. cit. 18: 177—214 (1987); 'Supplemento 3a', Bull. Soc. Linn. Provence 40: 73—110 (1989). [Keys; in Esperanto.].

Dobson, F.S. (2000). Lichens. An illustrated guide to the British and Irish species. 4th edn. Slough: Richmond Publishing. [Enlarged and revised edition with many colour photographs.]

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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