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## Review

# Fungi: Their role in deterioration of cultural heritage

Katja STERFLINGER\*

Department of Biotechnology, University of Natural Resources and Applied Life Sciences Vienna, Muthgasse 11, 1190 Vienna, Austria

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### ABSTRACT

Fungi play a considerable role for the deterioration of cultural heritage. Due to their enormous enzymatic activity and their ability to grow at low  $a_w$  values fungi are able to inhabit and to decay paintings, textiles, paper, parchment, leather, oil, casein, glue and other materials used for historical art objects. The weathering of stone monuments is significantly increased by epi- and endolithic fungi. In museums and their storage rooms, climate control, regular cleaning and microbiological monitoring are essential in order to prevent fungal contamination. Education and close collaboration of mycologists and restorers are needed to develop object specific methods for the conservation and treatment of contaminated objects.

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## 1. Introduction

Fungi – including yeasts, moulds, mushrooms and toadstools – have been playing a considerable role for human culture and the evolution of human society for thousands of years. Yeasts were used for beer and bakery products by the Egyptians, Kelts and Teutons – albeit not being aware of microbiological processes. Mushrooms probably served as food for hunters and gatherers since the beginning of humankind and toadstools as *Amanita muscaria* and species of *Psilocybe* were used as hallucinogens for cultic rites from Sibiria to South America. The psychedelic effect of LSD 45 derived from *Claviceps purpureum* influenced authors – Ernest Jünger, Aldous Huxley – and somehow even catalysed the so-called “cultural revolution” in the 1960s. A real revolution of invaluable benefit was the detection of the first antibiotic by Flemming in 1930 which

laid the basis for a completely new life-saving therapeutic approach. The history of fungi in medicine and their revolutionary impact in the cure of infectious diseases was recently highlighted by Wainwright (2008).

In contrast to their numerous beneficial effects, fungi also have their “dark side”: mycotoxins, pathogenicity, allergens, food spoilage and biodeterioration of materials. Biodeterioration of houses by mould was already mentioned in the bible as white, red or green “leprosy” or “fretting” on brick, clay and wood (Old Testament, Third Book of Moses, chapter 14, verses 33–57). Today, fungal contamination is an increasing problem not only in houses and working places. Objects of art in museums and their depots are seriously threatened by fungal contamination. The prevention of mould growth in museums as well as the development of appropriate treatment measures for contaminated objects is a challenge for

\* Corresponding author.

E-mail address: [katja.sterflinger@boku.ac.at](mailto:katja.sterflinger@boku.ac.at)

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restorers, museum curators and architects. This has implications for the techniques of cleaning and conservation of objects but also consequences for the occupational safety and health of restorers and other museum personnel. In outdoor environments fungi and lichen are the most important agents of biodeterioration of historic monuments and sculptures made of stone, mortar and plaster.

## 2. Fungi in the museum environment

Contamination of pieces of art presented in exhibition rooms or stored in depots and their spoilage by fungi is not exceptional but rather frequent in old and newly built museums (Allsopp et al., 2004; Nittérus, 2000a; Capitelli et al., 2009;

Manoharachary et al., 1997; Mesquita et al., 2009; Pangallo et al., 2007; Koestler et al., 2003). It is well known to mycologists that fungi are able to inhabit, to alter and to degrade all types of organic and inorganic materials (Fig. 1A–F). However, most conservators and museum curators are not aware of this enormous deteriorative potential.

Historically, pieces of art were made of all types of organic materials and these materials are again used for an authentic restoration or conservation of the objects in recent times: Paint was made of mineral pigments bound with organic binders such as egg yolk, casein, linseed-, poppy seed-, hempseed oil, Chinese wood oil or different resins. Linen canvas clamped on wooden frames serves as painting ground and was often primed with rabbit skin glue before painting. Gold



**Fig. 1 – (A, B) Part of a wood ceiling with 12th century paintings. The paint layer was deteriorated by *Aspergillus* sp. (C) Pastel painting with fungal contamination due to packing in plastic foil. (D) Mould on imperial Austrian horse trappings made of textiles. (E) Historical frames with gold leaf strongly contaminated by fungi. (F) Dense lawn of *Trichoderma* sp. on historical book.**

leaf on precious wooden or stucco frames was applied using organic glues, linseed or turpentine oil. Historic glues were based on cellulose or rabbit skin. Sculptures and other art objects carry décors made of textiles, leather, straw, clay, natural hair or feathers. The most precious documents of humankind are books and scrolls made of paper, papyrus and parchment. Because of the tremendous diversity of exoenzymes produced by fungi – cellulases, glucanases, laccases, phenolases, keratinases, mono-oxygenases and many more – and their remarkable ability to grow at low  $a_w$  values the preservation of museum objects is inevitably connected with prevention of mould, monitoring of mould and treatment of mould on contaminated objects.

A compilation of fungi frequently occurring on paper, paintings and other materials in museum is given in Table 1. The data are based on more than 20 studies carried out in Austrian museums since 2000 and on data collected from different case studies in the literature. Most fungi playing a role for deterioration of cultural heritage phylogenetically belong to the Euascomycetes; hemiascomycetes – yeasts – are rarely isolated from art objects. Teleomorphs are rarely found and the only teleomorph genera that frequently occur are *Chaetomium* – mostly on paper, wood and feathers – and *Eurotium* – in environments with low  $a_w$  values. Occurrence of basidiomycetes is restricted to wood degradation in churches or other protected historical monuments. Zygomycetes are frequently isolated from pieces of art but in most cases they can be regarded as transients not being really established on the objects.

Fungal growth on objects of cultural heritage often causes a serious aesthetical spoiling due to colony formation and fungal pigments (Sterflinger et al., 1999). Moreover, fungi degrade materials and thus affect objects substantially: the enzymatic degradation of organic binders causes reduction or even loss of paint layers (Fig. 1A–C). Fungi penetrate cracks and migrate underneath paint layers thus causing detachment. In paper conservation fungi are a special problem due to their ability to excrete cellulases (Fig. 1F). Lignin degrading fungi are rarely observed in indoor environments, but considerable damage can be caused by the cellulose degraders *Serpula lacrymans* or *Conophora puteana* in churches and other objects of cultural heritage if wooden altars or the roof structures are attacked. Also originals and museum reconstructions of historical buildings are considerably damaged by *S. lacrymans* (Bech-Andersen and Elborne, 2004).

The development of fungi in museums is to a large extent determined by the indoor climate, the amount of available nutrients – from the atmosphere and from the materials themselves – and also by the cleaning intervals in the museum. The indoor climate as indicated by temperature, relative humidity and by specific humidity is the most important factor for fungal growth. It is also closely related to the buildings physical properties, especially the thermal insulation and the tendency to generate condensate from warm indoor air on cold walls of the building envelope (Camuffo, 1998). Depending on the climate in the museum or storage rooms the fungal diversity is restricted to few xerophilic and xerotolerant species such as *Eurotium* sp., *Aspergillus* sp. or *Wallemia* sp.. Only in storage rooms where the humidity is raised to more than 70 % for a period of several weeks or month is a high fungal diversity able to establish. The climate ranges allowing fungal spores

to germinate and that restrict the growth of the fungal mycelium are shown in the isopleth systems by Sedlbauer and Krus (2003). The authors also show that hygroscopic materials support the growth of fungi at low relative humidity and that the water demand depends on the biodegradability of the substrate. The objects influence the development of the fungal community by their chemical composition and biodegradability for species with different exo-enzymes.

In museums the range of 55 % RH is generally regarded as the border line for fungal growth and thus climate control is adjusted below this value. In fact, fungi that are able to survive at a relative humidity of 55% are rare and restricted to extreme environments such as hot and cold deserts. So why do mesophilic hyphomycetes frequently occur in museums? All museums in the world measure temperature and humidity in storage and exhibition rooms by means of modern data logger, data writers or simple hygro- and thermometers. However, the way and location of climate measurements are often insufficient to reflect the real climate and to detect different climatic zones in the building. In his book on microclimate in museums Camuffo (1998) illustrates the complexity of climate monitoring that cannot just be monitored by a single data logger in the middle of a museum room. The influence of air stream through doors, warming by sunlight and daily changes of temperature gradients as well as the isolation and exposition of the building envelope have to be considered as important factors. In fact, fungal growth mostly happens between shelves with little aeration or near to walls with temperatures below the dew point (Fig. 2E, F). Micro-niches are often also created by wrapping of single objects into plastic foils or extremely tight boxes not allowing an exchange of air and vapour (Figs 1C, 2C, D).

The fungal micro-flora in museums is also influenced by the atmospheric particular matter carrying carbonates, minerals and others. Gysels et al. (2004) have shown in a study on the Royal Museum of Fine Art in Antwerp that the indoor aerosols were largely determined by the outdoor atmosphere and the outdoor sources of organic and inorganic pollutants. Fungi are well able to degrade different types of organic pollutants including polycyclic aromatic hydrocarbons (PAHs). Therefore the fungal diversity on monuments in an urban environment was found to be much higher than in a rural environment of the climatic zone (Sterflinger and Prillinger, 2001).

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### 3. Fungi as deteriorative agents of stone monuments

From the biological point of view stone is an extreme environment poor of nutrients, with enormous changes of humidity, with mechanical erosion due to wind and rain and high doses of UV radiation. Nevertheless, stone is inhabited by fungi and other microorganisms in all climate regions of the earth (Sterflinger, 2005; Selbmann et al., 2005). Epilithic fungi – living on the rock – and endolithic fungi – living inside of pores and fissures – fungi play a major role in the weathering of monuments made of rock. Fungi might be the most important endoliths on building stone, on mortar and on plaster because their activity is high and they are extremely erosive (Sterflinger, 2000; Scheerer et al., 2009; Gadd, 2007). On monuments there



**Table 1 – Most frequent hyphomycetes in museums and on materials of objects of art<sup>a, b, c, d, e</sup>**

Substrate	Genus/species
Paintings: oil, water color, acrylic	<i>Alternaria</i> sp. <i>Aspergillus flavus</i> , <i>Aspergillus</i> sect. <i>Niger</i> , <i>A. sydowii</i> , <i>A. versicolor</i> <i>Aureobasidium pullulans</i> <i>Chaetomium funicola</i> <i>Cladosporium herbarum</i> , <i>C. cladosporioides</i> <i>Eurotium chevalieri</i> , <i>E. rubrum</i> <i>Fusarium</i> sp. <i>Mucor</i> sp. <i>Penicillium chrysogenum</i> , <i>P. citrinum</i> , <i>P. decumbens</i> and many other species of the genus
Paper (laid-paper, wood pulp paper) and cellulose textiles (cotton, linen)	<i>Alternaria</i> sp. <i>Aspergillus clavatus</i> , <i>A. flavus</i> , <i>A. glaucus</i> , <i>A. terreus</i> , <i>A. repens</i> , <i>A. ruber</i> , <i>A. fumigatus</i> , <i>A. ochraceus</i> , <i>A. nidulans</i> , <i>Aspergillus</i> sect. <i>Niger</i> <i>Botrytis cinerea</i> <i>Chaetomium globosum</i> , <i>C. elatum</i> , <i>C. indicum</i> <i>Eurotium amstelodami</i> <i>Fusarium</i> sp. <i>Mucor</i> sp. <i>Paecilomyces variotii</i> <i>Penicillium chrysogenum</i> , <i>P. funiculosum</i> , <i>P. pupurogenum</i> , <i>P. rubrum</i> , <i>P. variable</i> , <i>P. spinulosum</i> , <i>P. fellutatum</i> , <i>P. frequentans</i> , <i>P. citrinum</i> <i>Pichia guilliermondi</i> <i>Rhizopus oryzae</i> <i>Stachybotrys chartarum</i> <i>Toxicocladosporium irritans</i> <i>Trichoderma harzianum</i> , <i>T. viride</i> <i>Stemphiliium</i> sp. <i>Ulocladium</i> sp.
Parchment	<i>Cladosporium cladosporioides</i> <i>Epicoccum nigrum</i> <i>Phlebiopsis gigantea</i> <i>Penicillium chrysogenum</i> <i>Thanatephorus cucumeris</i>
Keratinous substrates (leather, wool, feathers, fur, hair)	<i>Absidia glauca</i> , <i>A. cylindrospora</i> , <i>A. spinosa</i> <i>Acremonium</i> sp. <i>Alternaria alternata</i> <i>Aspergillus sydowii</i> , <i>A. candidus</i> , <i>A. clavatus</i> , <i>A. carneus</i> , <i>A. foetidus</i> , <i>A. flavus</i> , <i>A. fumigatus</i> , and many other species of the genus <i>Arthroderma</i> sp. <i>Aureobasidium pullulans</i> <i>Chaetomium globosum</i> <i>Chrysosporium</i> sp. <i>Coniosporium</i> sp. <i>Cladosporium cladosporioides</i> <i>Cunninghamella echinulata</i> , <i>C. elegans</i> <i>Epicoccum nigrum</i> <i>Emericella</i> sp. <i>Geotrichum candidum</i> <i>Mucor</i> sp. <i>Penicillium brevicompactum</i> , <i>Penicillium chrysogenum</i> and many other species of the genus <i>Phoma medicaginis</i> <i>Scopulaiopsis</i> sp. <i>Stachybotrys chartarum</i> <i>Trichophyton</i> sp. <i>Rhizopus</i> sp.
Archeological findings: bones, ceramics	Archeological findings often carry a large load of spores, in case of contamination the diversity on the objects reflects the diversity of the respective soil

The identification of the fungi was carried out based on morphology and/or sequencing of the ITS1, 5.8 S, ITS2 region with subsequent homology search using the BLAST algorithm [<http://www.ncbi.nlm.nih.gov/BLAST/>].

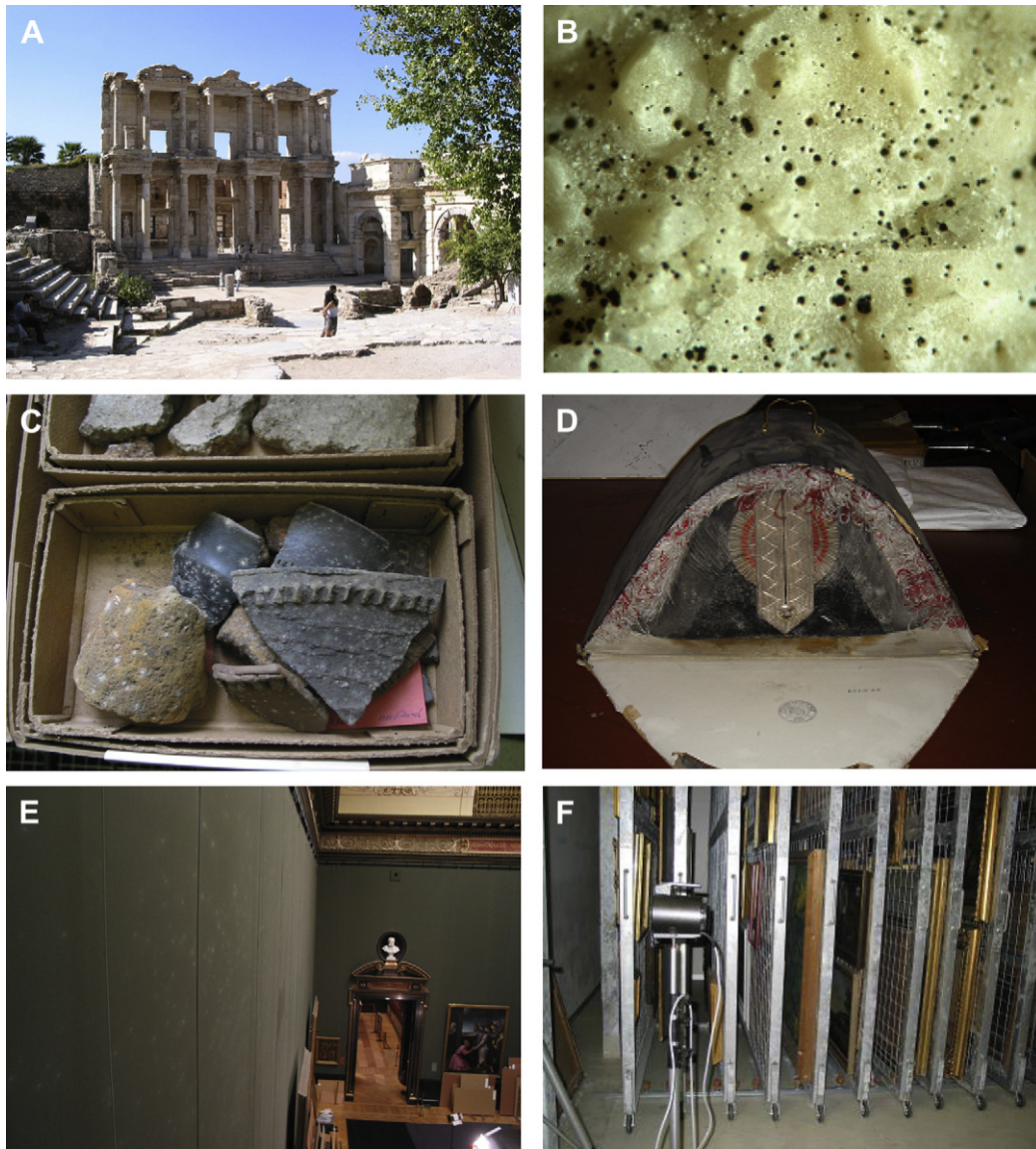
a Unpublished data Sterflinger/ACBR.

b Mesquita et al. (2009).

c Meier and Petersen (2006).

d Blyskal (2009).

e Pangallo et al. (2009).



**Fig. 2 – (A, B) Marble facade of the Celsus library in Ephesus (Turkey) with biopitting caused by microcolonial fungi. (C, D) Fungal contamination of archeological findings and historical helmets due to storage in tight cardboard boxes. (E) Fungal growth on textile tapestry on museum wall caused by wall temperature failing below the dew-point level. (F) The tight assembly of racks for storage of paintings does not allow sufficient ventilation and thus increases the risk of fungal growth.**

are two major morphological and ecological groups of fungi that are adapted to different environmental conditions. In moderate or humid climates the fungal communities are dominated by hyphomycetes including species of *Alternaria*, *Cladosporium*, *Epicoccum*, *Aureobasidium* and *Phoma*. In arid and semi-arid environments the fungal community shifts towards black yeasts and microcolonial fungi. Black fungi belonging to the genera *Hortaea*, *Sarcinomyces*, *Coniosporium*, *Capnobotryella*, *Exophiala* and *Trimmatostroma* form small black colonies on and inside the stone (Fig. 2A, B) and often occur in close association with lichen (Sterflinger, 2005). Famous monuments covered and deteriorated by fungi are the Acropolis of Athens, marble monuments of the Crimea and the antique temples of Delos (Diakumaku et al., 1995; Sterflinger,

2000). Due to their thick, melanized cell walls fungi resist also chemical attack and cannot easily be killed by biocides or other anti-microbial treatments. Black fungi dwell deep inside granite, calcareous limestone and marble and deteriorate those stones. The phenomenon of biopitting – the formation of lesions in a size range of up to 2 cm in diameter and depth on stone – is caused by the black fungi. Due to the strong melanization of the cell walls stones inhabited by these fungi appear spotty or are even completely covered by black layers. In addition to outdoor environments black fungi are also found on rock surfaces of caves and catacombs (Saarela et al., 2004). The oldest and most precious objects suffering from serious fungal invasions are rock art caves, especially the Lascaux cave (Bastian and Alabouvette, 2009).

#### 4. Prevention and treatments

The three “ounces of prevention” against fungal contamination in museums are: climate control, frequent cleaning and phenomenological monitoring. Climate concepts have to be developed taking into account the individual architecture of the museum. In spite of serious climate conditioning and monitoring, unexpected water damage frequently happens in old as well as new museum buildings. In this case the hygienic situation – namely the amount of dust carrying fungal spores – is decisive for the dimension of fungal contamination. Objects that are heavily loaded with fungal spores will easily be overgrown within a period of several days. However, a surface carrying a limited amount of spores will be much less affected. The air stream and air exchange in the rooms as caused by the ventilation system, the number of visitors, the action and air stream caused by the opening and closing of doors has quite some impact on the load of fungal spores carried inside a museum. New built storage rooms are now provided with filter systems to avoid the invasion fungal spores, plant pollen and dirt particles. Generally filter class F5–F7 is used as final filter in air conditioning systems for offices, sales rooms certain production plants and museums. Frequent cleaning using vacuum cleaners equipped with high efficiency particle absorbers (HEPA filters) is highly recommended to keep the spore load low.

Only recently important European museums have started to establish microbiological monitoring programmes including the measurement of fungal spores in the air by air sampling and the measurement of fungal spores on objects and museum shelves by surface contacts (Fig. 3A, B). Based on those measurements the hygienic status of the museum can be determined, concepts for optimizing the hygienic status and specific disaster management plans can be developed (Dicus, 2000; Barton and Wellheiser, 1985). Also the implementation of quarantine rooms for contaminated objects is increasing in museums and restoration studios.

In the process of restoration fungal spores and superficial mycelia will be removed mechanically following a careful investigation. The appropriate cleaning method is determined by the chemical composition and strength of the material itself and by the chemical quality of other than biogenic patinas. Testing the viability of the fungi is crucial for making the decision on further disinfection measures. Sampling of fungal spores or small fragments of mycelium using a needle (glass or surgical needle) is normally carried out directly in the museums. Malt Extract Agar and Dichlorane Glycerol agar are used routinely but a wide range of media might be used to test the potential of the fungi to decompose the material: cellulose agar, casein agar or stained indicator media (Pangallo *et al.*, 2009). On paper, pictures or photographs a defined part of the surface can be sampled with a scalpel or a sterile swab and being transferred to 0.9 % NaCl for transport to the lab (Fig. 3C–E). The NaCl and the swab itself can be embedded in suitable agar. In case the fungal flora turns out to be non-viable, disinfection is not necessary.

For disinfection of a recent and progressive fungal damage a limited range of physical and chemical methods are available (Allsopp *et al.*, 2004), only the most common of which

can be discussed here: the most effective physical method for killing fungi and their spores is the use of gamma radiation. Most authors report that the doses has to be higher than that normally used for bacteria and has to exceed 10–20 KGy for a complete killing of fungi (Nittérus, 2000a). However, gamma radiation might significantly affect the chemical composition of the cellulose fibres and thus the application has to be thoroughly considered. Chemical treatments include liquid biocides and fumigation with gases as methylbromide, ethylenebromide. The choice of the appropriate biocide is somehow restricted by the European Biocide directive (<http://ec.europa.eu/environment/biocides/index.htm>). Albeit a variety of biocides is still available on the market (Cooke, 2002) and in restoration three substance groups are approved to be effective against fungi with none or minimum irritation of the materials: (1) Products containing donors which slowly release formaldehyde. (2) Products containing quaternary ammonium compounds with an optimal chain length of C14–C16 (e.g. Metatin 5810-101, Neo Desogen, Dimanin, Antimoos). These so-called “quats” are rapid acting anti-microbials which are commonly regarded as relatively environment friendly. Their efficacy is reduced by high levels of salt or proteins. (3) Isothiazolinone, a more recent biocide, was documented to be effective and even preventive on paper objects. Treatments with different phenols (e.g. thymol, cymol) gave good results in several case studies but should not be regarded as general fungitoxic solutions. Ethanol (70 %), the most common disinfectant used in microbiology, can also have a good fungitoxic effect if the time of application is at least 2–3 min. Mere spraying of ethanol – as it is commonly carried out in restoration – is insufficient (Nittérus, 2000b).

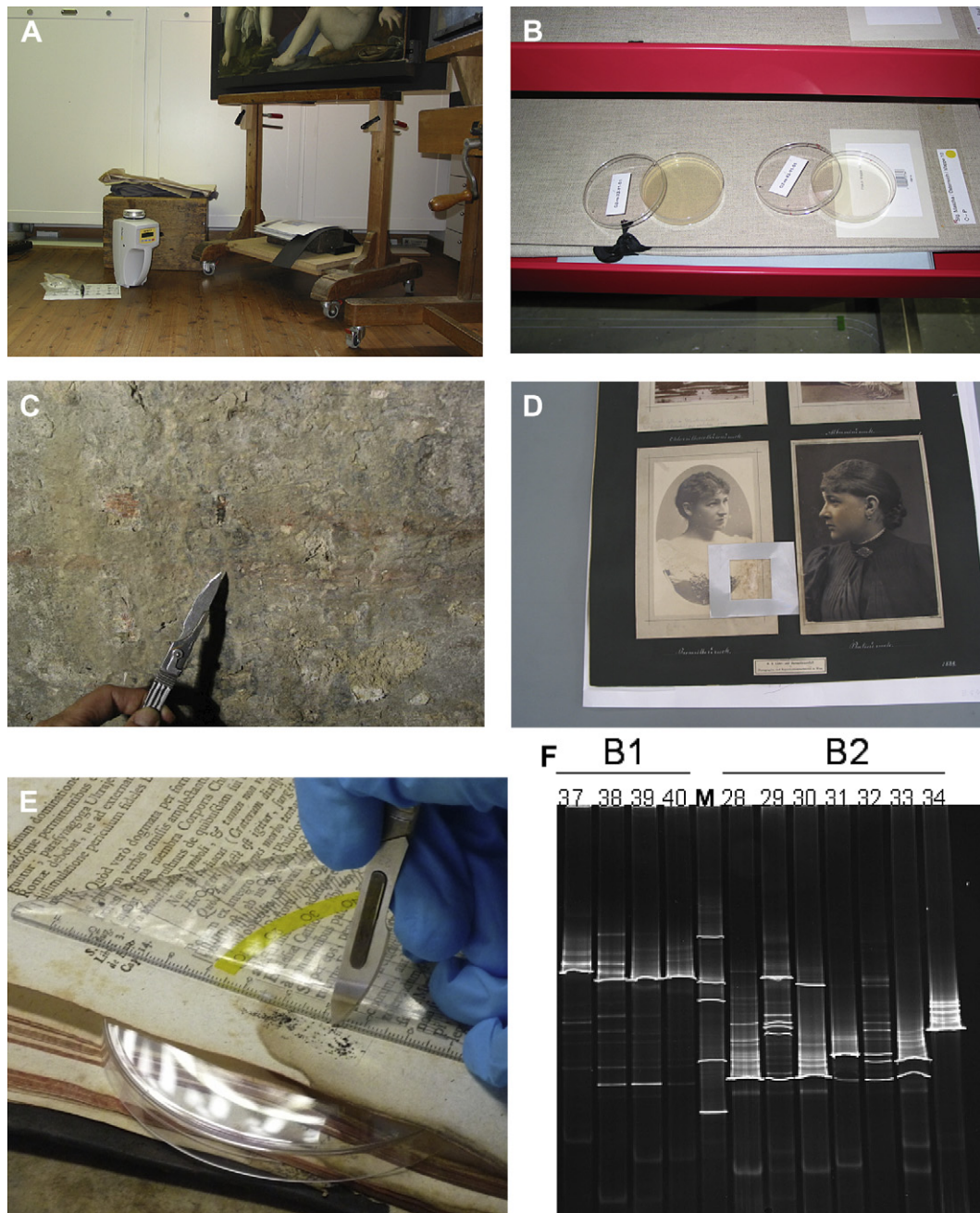
#### 5. Methods to study fungi on cultural heritage

Today, many sophisticated molecular techniques exist to study the interaction of microbes and materials. However, concerning the practice of conservation, consolidation and prevention of biodeterioration the phenomenological analysis of the objects using light and electron microscopy is the first and most important step (De los Rios and Ascaso, 2005).

Mycological research on biodeterioration in museums or monuments is still mainly based on classical cultivation methods using a variety of standardized media as MEA, DG18 and dichlorane rose bengale (DRBC) medium. In contrast to bacteria for which it is generally accepted that cultivation methods recover less than 1 % of the total present in environmental samples, the recovery rate concerning fungi is assumed to be more than 70 %. For this reason culture-based approaches are still extremely useful in mycology.

Nevertheless, the use of modern molecular techniques for the detection of fungi on and in materials of cultural heritage will provide a deeper insight and understanding of fungal community structures and their consequences for the material. Recently, the Internal Transcribed Spacers (ITS regions), which are nested in the nuclear rDNA repeat, have been selected to investigate the fungal diversity of fungi on building materials (Sterflinger and Prillinger, 2001; Martin and Rygielwicz, 2005). The ITS regions possess a high variation





**Fig. 3 – (A) Air sampling in a restoration studio carried out with MD8 air sampler. (B) Sedimentation plates for monitoring in a museum depot. (C) Sampling of medieval wall painting. (D) Sampling of photographs by swab within a marked area. (E) Sampling of fungal mycelium on a historical book. (F) DGGE fingerprint of a fungal population of contaminated library material showing remarkable differences between the books sampled (B1/B2) and between samples within books that were taken from different macroscopically visible deterioration phenomena (lanes 37–40 and 28–34).**

between taxonomically distinct fungal species and even within the species. ITS based analysis of fungi on paper from museums was carried out using Denaturing Gradient Gel Electrophoresis (DGGE) (Michaelsen *et al.*, 2006). For studies of microbial communities colonizing artworks, DGGE is the technique most often used (Portillo *et al.*, 2008). An example of a DGGE fingerprint from historical books is given in Fig. 3F.

Fluorescence *in-situ* hybridization FISH has also been applied in the field of conservation and restoration to study bacteria, archaea and fungi involved in the biodeterioration of surfaces (Urzi *et al.*, 2003). Furthermore, the application of FISH directly on adhesive tape strips added another advantage to this non-destructive sampling method: the identification “*in situ*” of the microorganisms present on a given area, without the destruction of the valuable surfaces and with little

biofilm disturbance. Since FISH, using DNA probes, is often hampered by rigid fungal cell walls, only recently peptide nucleic acid (PNA) were applied for fluorescent in-situ detection of filamentous fungi (Teertstra *et al.*, 2004). PNA probes are synthetic DNA mimics, where the negatively charged DNA backbone is replaced by a neutral polyamide backbone (Stender *et al.*, 2002). Due to this, PNA probes have better binding features to complementary targets and penetrate fungal cell walls more easily. This method could be a promising tool for specific detection and visualization of fungi on and in materials.

Recent improvements in molecular studies have shown the advantages of RNA-based molecular analyses. In an RNA-based approach, not only the presence of a fungal species but also its metabolic activity could be determined, since the levels of RNA in a cell are proportional to the need of that cell for synthesizing proteins required for metabolism. RNA-based studies have been carried out to investigate the yellow and grey colonizations on the walls of Altamira Cave, Spain (Portillo *et al.*, 2008). Also the analysis of the fungal proteome (2-D electrophoresis or metaproteomics) can bring new insights into the activity of fungi on art objects.

## 6. Conclusions

Fungi play a tremendous role in deterioration of our cultural heritage. This holds true for museum objects as well as for stone monuments in all climate zones of the earth. One important reason why fungi are a great problem for conservation of cultural heritage is a lack of information and training for restorers, curators and other museum personnel. There is a high demand for mycologists and microbiologist able to teach and to consult restorers and museum personnel. A basis for this is a transdisciplinary approach in teaching and the mycologists interest in material sciences and conservation. This however is a big challenge for mycologists and microbiologist but also a very special field of working that offers interesting insights into art and material sciences. It is worth emphasizing the special role of fungal culture collections where scientists have an extensive knowledge on fungal taxonomy and ecology. Although mycologists knowledge would be of tremendous value for museums, only very few collections offer consulting in this field. To my knowledge only one collection of those recognized by the World Federation of Culture Collections, namely the Austrian Center of Biological Resources and Applied Mycology ([www.boku.ac.at/acbr.html](http://www.boku.ac.at/acbr.html)) offers a special service for museums and other institutions working on care of monuments and cultural heritage.

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