



Grana

ISSN: 0017-3134 (Print) 1651-2049 (Online) Journal homepage: <http://www.tandfonline.com/loi/sgra20>

Fungal biodeterioration in historic buildings of Havana (Cuba)

Teresa Irene Rojas , María Jesús Aira , Amado Batista , Isara Lourdes Cruz & Sergio González

To cite this article: Teresa Irene Rojas , María Jesús Aira , Amado Batista , Isara Lourdes Cruz & Sergio González (2012) Fungal biodeterioration in historic buildings of Havana (Cuba), Grana, 51:1, 44-51, DOI: [10.1080/00173134.2011.643920](https://doi.org/10.1080/00173134.2011.643920)

To link to this article: <http://dx.doi.org/10.1080/00173134.2011.643920>



Published online: 13 Mar 2012.



Submit your article to this journal [↗](#)



Article views: 256



View related articles [↗](#)



Citing articles: 4 View citing articles [↗](#)

Fungal biodeterioration in historic buildings of Havana (Cuba)

TERESA IRENE ROJAS¹, MARÍA JESÚS AIRA², AMADO BATISTA¹, ISARA LOURDES CRUZ¹ & SERGIO GONZÁLEZ¹

¹Department of Microbiology and Virology, University of Havana, Havana, Cuba, ²Department of Botany, University of Santiago, Santiago de Compostela, Spain

Abstract

The incidence of fungi inside certain historic buildings and premises used for storing heritage collections in Havana (Cuba) and fungal contamination of the items stored there have been assessed. Cultivable airborne fungi were sampled using the slit-to-agar impaction method with a type S Chirana aeroscope; various substrates were swab-sampled in addition. Most common were *Aspergillus*, *Penicillium* and *Cladosporium*. The physiological features of some of the fungal strains isolated from the substrates were examined in order to evaluate their potential for biodeterioration. Numerous *Aspergillus* strains were able to produce cellulase and acids and to hydrolyse gelatine, while *Cladosporium* displayed the greatest ability to produce polyphenol oxidases. One third of the strains tested, all belonging to the genera *Aspergillus* and *Penicillium*, produced pigments.

Keywords: *fungi, volumetric air sampling, swab, cultural heritage*

In tropical climates, where prevailing heat and humidity conditions favour growth and sporulation, elevated airborne propagule concentrations can increase the risk of biodeterioration, particularly in indoor environments (Borrego et al., 2010). Unlike other microbial groups, saprophytic fungi can colonise a wide range of substrates and ecological niches; the extent of that colonisation depends on the species involved, on their genetic potential and on prevailing environmental conditions (Nugari & Roccardi, 2001; Lugauskas et al., 2003).

The most common fungi in indoor environments belong to the genera *Aspergillus*, *Penicillium* and *Cladosporium*, and to a lesser extent to *Fusarium*, *Trichoderma*, *Alternaria*, *Curvularia* and *Paecilomyces*; many of these are known to cause biodeterioration. Species of the ascomycete *Chaetomium* may degrade various polymers and are often found growing on cellulose-containing substrates (Abín et al., 2002; Portugal et al., 2009).

Fungi can prompt physical biodeterioration through mycelial growth and chemical biodeterioration through the action of their metabolic products

(including organic acids, mycotoxins and pigments). Biodeterioration may not always be visible to the naked eye, but affects the internal structure of the substrate (Ranalli et al., 2009). Changes in colour, perhaps the most common manifestation of fungal contamination, may be a pointer to eventual structural damage due to the considerable capacity of many fungi to degrade a whole range of organic compounds (Florian, 2003).

Mycological research on indoor environments in Cuba to date has pursued a number of very specific aims (Sánchez et al., 1988; Vaillant et al., 1989; Aira et al., 2002; Rojas et al., 2002; Borrego et al., 2008, 2010). Published studies have identified the most common genera and/or species, but have not addressed the whole mycobiota; as a result, the fungi present in storage areas and on stored items have yet to be fully characterised. This is an essential first step towards the adequate conservation of any building or collection.

The present study sought to evaluate the incidence of fungi inside certain historic buildings and premises used for storing heritage collections in

Havana (Cuba) and to assess fungal contamination of the items stored there, with a view to characterising the mycobiota and estimating its capacity for biodeterioration, as a prelude to recommending preventive conservation measures.

Material and methods

Sampling and biological material

Sampling was carried out in seven historic buildings in Havana: the Central Library at the University of Havana, the Montané Museum, the Tomás Roig Museum, the Felipe Poey Museum and three buildings used to store collections. These premises house important documents, books, paintings, photographs and items of Pre-Columbian culture, along with the personal possessions of historians and scientists of earlier centuries, and invaluable biological collections.

Cultivable airborne fungi were sampled using a slit-to-agar impaction type S Chirana aeroscope (Chirana s.r.o., Piešťany, Slovakia) with a suction capacity of 29 litres of air/minute. This method is comparable to conventional techniques for viable spore sampling; previous studies showed that one minute with this flow rate is enough to prevent an overload of propagules on the agar plate (Rojas et al., 2008). Substrates (including wood, glass, paper and textiles) were sampled using sterile swabs moistened with Tween 80[®] saline solution; spores were grown on malt extract agar with 0.05 g/l chloramphenicol.

Samplings were carried out from 2005 to 2010; the collection of airborne fungal propagules and swab sampling were simultaneously done for each site with the purpose to obtain more information of the existent mycobiota. Samples were taken at a height of one metre using a diagonal design with three, five or seven collection points according to the surface of each site, following the criteria established for the FEDECAI (2007). Three replications were undertaken at each sampling point and the results were then averaged. The temperature and relative humidity were also measured in each sampling point of every location.

Plates were incubated at $28 \pm 1^\circ\text{C}$ and at $37 \pm 1^\circ\text{C}$ for five days and colonies were re-sown in Czapek medium and malt extract agar (Atlas, 2000). Visibly-damaged textile samples were analysed using a conventional enrichment method (Desai & Betrabet, 1972).

Items were selected for analysis on the basis of their cultural, scientific or economic value and the extent of biodeterioration. Sampled materials contained cellulose (books, documents and textiles), lignocellulose (wooden items, glass-case frames, cabinets and packing cases) and inorganic matter (glass cases

and boxes). Some cellulosic items also contained protein-based substances including gelatine and casein, both widely used in the manufacture of photographic paper and, in earlier times, for the production of canvases for painting.

Following an analysis of genus diversity, species identification was performed for the most abundant genera. Isolates collected by swab-sampling were also identified at species level if they belonged to genera with known biodeterioration potential. A total of 77 strains were selected from monospore cultures of these species in order to evaluate their capacity for biodeterioration; every effort was made to ensure that each species was represented by at least one strain isolated from each type of substrate.

Physiological analysis

Capacity to produce cellulase, polyphenol oxidase (laccase and/or peroxidase), acids and pigments was evaluated in all strains; additionally, proteolytic capacity was evaluated in *Aspergillus* strains and in species isolated from protein-containing materials. Cellulolytic capacity was evaluated following Borrego et al. (2010) using filter paper as sole carbon source. Cultures were incubated for 21 days, substrate degradation was checked regularly. A *Chaetomium globosum* strain (CCMFB-101) was used as positive control.

Polyphenol oxidase production capacity was evaluated following Pointing (1999), using a malt extract assay medium containing tannic acid. Plates were incubated for seven days. Positive strains displayed a dark halo or area of diffusion around the colony, indicating the presence of these enzymes. A *Lentinus hirtus* strain (CCMFB-B8) was used as positive control.

To evaluate acid production, strains were grown for seven days on Czapek medium containing a phenol-red-pH indicator (Klich & Pitt, 1994). The positive control was an *Aspergillus niger* strain (CCMFB-O5), reported by Abín et al. (2002) to be an acid producer. A change in the colour of the assay medium from red to yellow was regarded as a positive result; strains in which that colour change took place at $\text{pH} < 7$ were deemed positive.

Pigment production was also analysed using Czapek medium. Strains showing any colouring after incubation for seven days were considered positive. The ISCC-NBS (1964) colour chart was used to ensure uniformity of criteria.

Proteolytic capacity was evaluated using both gelatine and casein substrates. For gelatine hydrolysis, strains were inoculated onto Czapek medium at pH 6.5 and 8, containing 15% gelatine as sole source of carbon and nitrogen (Abrusci et al., 2005). After

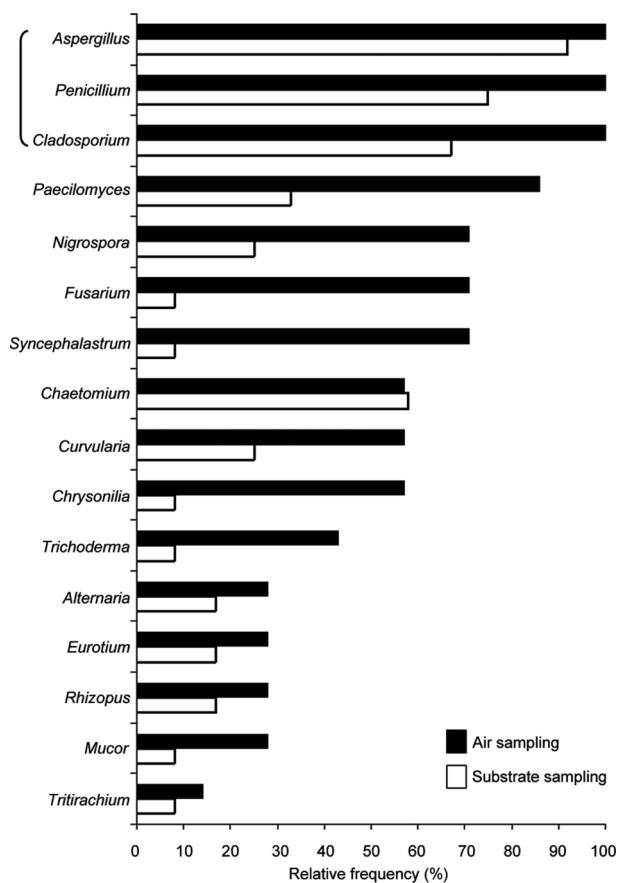


Figure 1. Relative frequency of the fungal genera identified in the air (Chirana aeroscope) and on substrates (swab).

incubation for seven days, assays were considered positive if, after one hour at 4–5 °C, the culture medium had not solidified. If results were negative, incubation was continued, and results were checked at 15, 21 and 30 days using the same procedure. An *Aspergillus niger* strain (CCMFB-A20) known to degrade gelatine at both pH levels was used as positive control. For casein hydrolysis, strains were inoculated onto Czapek medium at pH 6.5 and 8, containing 2% casein as sole source of carbon and nitrogen (Herrera, 1985). Proteolytic capacity was confirmed by the appearance of a hydrolysis halo around the colony after incubation for seven days. An *Aspergillus flavus* strain (CCMFB-105), known to hydrolyse casein at both pH levels, was used as positive control.

Three replicates were performed for each analytical test. Cultures were incubated at 28 ± 1 °C, with varying incubation time in each case. All positive control strains were retrieved from the fungal culture collection maintained by the Biology Faculty of the University of Havana (CCFB) whose degrading capacity had previously been verified. A sample grown on uninoculated medium was used as negative control in each case.

Results

The most common genera in the air and on substrates were *Aspergillus* (100% versus 92%, respectively), *Penicillium* (100% versus 75%) and *Cladosporium* (100% versus 67%) as shown by the analysis of relative frequencies. The other genera identified including *Chaetomium*, *Paecilomyces*, *Curvularia*, *Nigrospora*, *Alternaria*, *Eurotium*, *Rhizopus*, *Chrysonilia*, *Fusarium*, *Mucor*, *Syncephalastrum*, *Trichoderma*, *Tritirachium* and *Zygosporium*, displayed relative frequencies between 14% and 86% in the air and 8% and 55% on substrates (Figure 1).

Fungal diversity was greater in the air than on the objects sampled (28 versus 18 genera, respectively). *Aureobasidium*, *Bipolaris*, *Chaetosartorya*, *Emericella*, *Cephalosporium*, *Cunninghamella*, *Humicola*, *Monocillium*, *Pithomyces*, *Periconia*, *Stachybotrys* and *Staphylotrichum* were isolated only in the air. Most fungi collected by swabbing (88%) were isolated from cellulose-containing material, 39% were isolated from lignocellulose-containing and/or protein-containing items; 17% came from inorganic matter. In terms of sampling sites, diversity was greatest in the Central Library and one of the storage buildings, where 11 and 10 genera, respectively, were identified.

Species identification was performed for strains belonging to the genera most commonly isolated on stored objects and those associated with elevated degrading potential. Of the 38 species identified, 13 belonged to the genus *Aspergillus* (i.e., *A. caespitosus*, *A. candidus*, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. oryzae*, *A. sydowii*, *A. tamarii*, *A. terreus*, *A. versicolor* and the two teleomorphs *Eurotium chevalieri* and *Emericella nidulans*); seven to *Penicillium* (i.e., *P. chrysogenum*, *P. citrinum*, *P. corylophilum*, *P. glabrum*, *P. janthinellum*, *P. purpurogenum*, *P. simplicissimum*); four to *Cladosporium* (i.e., *C. cladosporioides*, *C. herbarum*, *C. oxysporum*, *C. sphaerospermum*); three to *Chaetomium* (i.e., *C. bostrychodes*, *C. globosum*, *C. indicum*); two to *Curvularia* (i.e., *C. lunata*, *C. pallens*) and two to *Rhizopus* (i.e., *R. oryzae*, *R. stolonifer*). Other genera were represented by single species (*Alternaria alternata*, *Chrysonilia sitophila*, *Fusarium oxysporum*, *Mucor circinelloides*, *Nigrospora sphaerica*, *Paecilomyces variotii* and *Syncephalastrum racemosum*).

Physiological properties have been characterised for the 77 selected strains (Tables I, II). Cellulase production was recorded for 87% of the strains of the studied fungi and was particularly consistent in *Aspergillus* species, the only exceptions being *Emericella nidulans* on textiles, *A. oryzae* on photographs and *A. candidus* on wood. Ability to produce polyphenol oxidases was found only for *Cladosporium* species (i.e., *C. cladosporioides*, *C. herbarum* and

Table I. Physiological analysis of the fungal strains in cellulosic substrates.

Strains	Paper substrates				Textile substrates			
	Cellulase	Polyphenol oxidase	Acid	Pigments	Cellulase	Polyphenol oxidase	Acid	Pigments
<i>Aspergillus fumigatus</i>	+	-	-	-				
<i>Aspergillus flavus</i>	+	-	+	-	+	-	+	Reddish
<i>Aspergillus niger</i>	+	-	+	-	+	-	+	Yellow
<i>Aspergillus oryzae</i>					+	-	+	-
<i>Aspergillus sydowii</i>	+	-	+	-	+	-	+	Reddish
<i>Aspergillus tamaritii</i>	+	-	+	-	+	-	+	-
<i>Aspergillus terreus</i>	+	-	+	Amber	+	-	+	Amber
<i>Aspergillus terreus</i>	+	-	+	Amber				
<i>Aspergillus versicolor</i>					+	-	+	Yellow
<i>Aspergillus versicolor</i>					+	-	+	Orange
<i>Eurotium chevalieri</i>					+	-	+	Brown
<i>Emericella nidulans</i>					-	-	+	-
<i>Penicillium chrysogenum</i>	+	-	+	Yellow	+	-	-	-
<i>Penicillium citrinum</i>	+	-	+	Yellow	+	-	+	Yellow
<i>Penicillium corylophilum</i>	+	-	+	-	+	-	+	-
<i>Penicillium glabrum</i>	+	-	+	Yellow	+	-	+	Yellow
<i>Penicillium janthinellum</i>	+	-	+	-				
<i>Penicillium purpurogenum</i>					+	-	+	Reddish
<i>Penicillium simplicissimum</i>					+	-	+	Reddish
<i>Cladosporium cladosporioides</i>	+	-	+	-	+	-	+	-
<i>Cladosporium herbarium</i>					+	-	+	-
<i>Cladosporium oxysporum</i>	+	-	+	-	+	-	+	-
<i>Cladosporium sphaerospermum</i>	+	-	+	-				
<i>Chaetomium bostrychodes</i>	+	-	+	-				
<i>Chaetomium globosum</i>	+	-	+	-	+	-	+	-
<i>Chaetomium indicum</i>	+	-	+	-				
<i>Chrysonilia sitophila</i>	+	-	-	-				
<i>Curvularia lunata</i>	+	-	-	-	+	-	+	-
<i>Mucor circinelloides</i>					-	-	+	-
<i>Nigrospora sphaerica</i>	+	-	+	-	-	-	+	-
<i>Rhizopus oryzae</i>	-	-	-	-				
<i>Rhizopus stolonifer</i>	-	-	-	-				
<i>Syncephalastrum racemosum</i>	+	-	+	-				
<i>Trichoderma</i> sp.					+	-	+	-
<i>Tritirachium</i> sp.					+	-		-
<i>Zygosporium</i> sp.	-	-	-	-				

Legend: + positive, - negative.

C. oxysporum) and for *Curvularia pallescens*, all isolated from wooden items.

Acid production was observed for 87% of strains, and was again particularly marked in *Aspergillus* species, with the exception of *A. fumigatus* on cellulose and protein-containing material and *A. clavatus* and *A. sydowii* on wood; acid production was also found for *Penicillium chrysogenum* on textiles, *P. glabrum* on protein-containing material and *Alternaria tenuissima* on wood.

Pigment production was recorded for 33% of the strains tested, all belonging to the genera *Aspergillus* and *Penicillium*. Yellow pigment predominated for both genera and was more abundant amongst strains isolated from textiles.

All *Aspergillus* strains hydrolysed gelatine at pH 6.5, while 87% did so at pH 8; fewer strains hydrolysed casein (Table III). *Penicillium chrysogenum* and *P. glabrum* strains hydrolysed gelatine and casein

at both pH levels, while *Cladosporium oxysporum* hydrolysed casein at both pH levels but gelatine only at pH 8.

Discussion

Sixteen of the identified genera were found both in the air and on stored items in the buildings evaluated; the predominant genera in both cases were *Aspergillus*, *Penicillium* and *Cladosporium*. Fungal diversity was greater in the air than on the substrates sampled. Earlier studies in Cuba have reported similar results from museums, archives and libraries at the University of Havana (Rojas et al., 2002), in storage areas containing mostly cellulose-based materials such as the National Archive (Vaillant et al., 1989, Borrego et al., 2008), on photographic materials (Borrego et al., 2010) and inside churches (Sánchez et al., 1988) and homes (Aira et al.,

2002). Research in other parts of the world has also highlighted the prevalence of *Aspergillus*, *Penicillium* and *Cladosporium* as well as *Alternaria* in religious buildings (Aira et al., 2007) and their effect on frescoes (Nugari & Roccardi, 2001) and historical documents (Robledo & Moretti, 1986) has been documented. Studies in modern buildings with problems of damp have stressed the adverse effects of *Penicillium* on wood and inorganic material and of *Penicillium* and *Cladosporium* on paper (Hyvärinen et al., 2001). *Penicillium chrysogenum* has been isolated from black and white cine film in the Spanish film archives (Abrusci et al., 2005).

Research of this kind has a twofold value. It contributes both to the conservation of cultural heritage and to the safeguarding of human health. Many fungi pose health risks, causing not only frequent allergic diseases but also immunotoxic diseases such as sick building syndrome (Burch & Levetin, 2002). Indoor fungal spores are strongly linked to respiratory diseases (Stryjakowska-Sekulska et al., 2005) and there is considerable evidence to suggest that elevated spore counts inside buildings can prompt allergy symptoms (Peltola et al., 2001). Some of the genera isolated in this study such as *Stachybotrys* are widely associated with symptoms of ill health in exposed people (Salkinoja-Salonen et al., 2003) and biodeterioration of damp materials (Kasznicza-Kocot et al., 2007).

This study focussed on issues linked to heritage conservation. Fungi can colonise almost everything, given favourable conditions of growth, and their spores can remain viable for hundreds of years. Evaluation of the strains isolated here from various substrates confirmed the considerable enzyme potential of *Aspergillus*, *Penicillium* and *Cladosporium*, as also reported by Villalba et al., 2004. Most of the strains tested displayed capacity for cellulase production, which was most remarkably in *Aspergillus* species particularly in those isolated from paper and textiles, including *A. niger*, *A. terreus*, *A. fumigatus*, *A. tamaritii*, *A. oryzae*, *A. versicolor* and *A. flavus*. Some reports testified the potential of these fungi for the biodeterioration of such substrates (Das et al., 1997; Borrego et al., 2010).

Other species exhibiting biodeterioration potential were *Chaetomium globosum* and *C. indicum* isolated from paper and textiles, *Fusarium oxysporum* isolated from protein-containing material, *Trichoderma* from textiles and several *Penicillium* species. *Penicillium chrysogenum* was present on all substrates and displayed remarkable cellulolytic capacity on all of them. This potential stems from the presence of a complete cellulase enzyme system able to partially or wholly break down cellulose into cellobiose and glucose (Ovando-Chacon &

Table II. Physiological analysis of the fungal strains in protein, lignocellulosic and inorganic substrates.

Strains	Polyphenol			Pigments
	Cellulase	oxidase	Acid	
Protein substrates				
<i>Aspergillus flavus</i>	+	–	+	–
<i>Aspergillus niger</i>	+	–	+	Yellow
<i>Aspergillus oryzae</i>	–	–	+	–
<i>Aspergillus fumigatus</i>	+	–	–	–
<i>Penicillium chrysogenum</i>	+	–	+	Yellow
<i>Penicillium glabrum</i>	+	–	–	Yellow
<i>Nigrospora sphaerica</i>	+	–	+	–
<i>Fusarium oxysporum</i>	+	–	+	–
Lignocellulosic substrates				
<i>Aspergillus caespitosus</i>	+	–	+	Purple
<i>Aspergillus candidus</i>	–	–	+	–
<i>Aspergillus clavatus</i>	+	–	–	Light brown
<i>Aspergillus fumigatus</i>	+	–	+	–
<i>Aspergillus niger</i>	+	–	+	Yellow
<i>Aspergillus sydowii</i>	+	–	–	Reddish
<i>Aspergillus tamaritii</i>	+	–	+	–
<i>Penicillium chrysogenum</i>	+	–	+	Yellow
<i>Cladosporium cladosporioides</i>	–	+	+	–
<i>Cladosporium cladosporioides</i>	+	+	+	–
<i>Cladosporium herbarum</i>	+	+	+	–
<i>Cladosporium oxysporum</i>	+	+	+	–
<i>Cladosporium sphaerospermum</i>	+	–	+	–
<i>Curvularia pallescens</i>	+	+	+	–
<i>Alternaria tenuissima</i>	+	–	–	–
<i>Paecilomyces variotii</i>	–	–	+	–
Inorganic substrates				
<i>Aspergillus clavatus</i>	+	–	+	–
<i>Aspergillus flavus</i>	+	–	+	–
<i>Penicillium chrysogenum</i>	+	–	+	Yellow

Legend: + positive, – negative.

Waliszewski, 2005). Valentín (2003) reports that these powerful cellulolytic species cause deterioration of documents and other plant- and textile-based cultural property. Although Thakre and Bhajbhujje (1991) have highlighted the involvement of *Paecilomyces* in the biodeterioration of books and journals, the *Paecilomyces variotii* strain tested here displayed no cellulolytic capacity; it may, certainly, have other complex enzymes not tested here.

Table III. Protelolytic ability of the fungal strains in cellulosic, lignocellulosic, protein and inorganic substrates.

Strains	Gelatine pH 6.5	Gelatine pH 8	Casein pH 6.5	Casein pH 8
Cellulosic substrates				
<i>Aspergillus flavus</i>	+	+	+	+
<i>Aspergillus fumigatus</i>	+	+	-	+
<i>Aspergillus niger</i>	+	+	+	+
<i>Aspergillus sydowii</i>	+	+	+	+
<i>Aspergillus tamaritii</i>	+	+	+	+
<i>Aspergillus terreus</i>	+	+	+	-
<i>Aspergillus terreus</i>	+	+	+	+
<i>Aspergillus flavus</i>	+	+	+	+
<i>Aspergillus niger</i>	+	+	-	+
<i>Aspergillus oryzae</i>	+	+	+	+
<i>Aspergillus sydowii</i>	+	+	+	+
<i>Aspergillus tamaritii</i>	+	+	+	+
<i>Aspergillus terreus</i>	+	+	+	-
<i>Aspergillus versicolor</i>	+	-	+	-
<i>Aspergillus versicolor</i>	+	-	+	-
<i>Eurotium chevalieri</i>	+	-	+	-
<i>Emericella nidulans</i>	+	+	+	-
Lignocellulosic substrates				
<i>Aspergillus caespitosus</i>	+	+	+	+
<i>Aspergillus candidus</i>	+	+	+	+
<i>Aspergillus clavatus</i>	+	+	+	+
<i>Aspergillus fumigatus</i>	+	+	-	+
<i>Aspergillus niger</i>	+	+	-	+
<i>Aspergillus sydowii</i>	+	+	+	+
<i>Aspergillus tamaritii</i>	+	+	+	+
Protein substrates				
<i>Aspergillus flavus</i>	+	+	-	+
<i>Aspergillus fumigatus</i>	+	+	-	+
<i>Aspergillus niger</i>	+	-	+	-
<i>Aspergillus oryzae</i>	+	+	+	-
<i>Cladosporium oxysporum</i>	-	+	+	+
<i>Penicillium chrysogenum</i>	+	+	+	+
<i>Penicillium glabrum</i>	+	+	+	+
Inorganic substrates				
<i>Aspergillus clavatus</i>	+	+	+	+
<i>Aspergillus flavus</i>	+	+	-	-

Legend: + positive, - negative.

Production of polyphenol oxidases on lignocellulose-containing substrates was found only for three *Cladosporium* species (*C. cladosporioides*, *C. herbarum* and *C. oxysporum*) and for *Curvularia pallescens*; all were isolated from wooden items. White-rot basidiomycetes are the only organisms known to wholly degrade lignin; degradation of lignocellulose-containing substrates by many other micromycetes is limited. However, some soft-rot *Aspergillus*, *Penicillium* and *Fusarium* species secrete cellulase that breaks down cellulose and, to a lesser

extent, lignin; moreover, brown-rot has been reported by Sánchez et al. (1988) in *A. flavus* and *Trichoderma lignorum* isolated from wood. Isolation of *Cladosporium herbarum*, *Cladosporium oxysporum* and *Curvularia pallescens* from lignocellulose-containing substrates has not previously been reported in Cuba; the strains tested here were found to produce polyphenol oxidases.

The ability of fungi to produce organic acids and pigments plays a crucial role in the discolouration and degradation of different materials in cultural heritage objects. Acid production was found for the vast majority of strains tested, including all *Aspergillus* strains except *A. fumigatus* (isolated from cellulose- and protein-containing material) and both *A. clavatus* and *A. sydowii* (isolated from wood). These acids form calcium salts or act as chelating agents of mineral cations, favouring the biodeterioration process. Ljaljevic and Vukojevic (2009) have reported on the deterioration caused by *Aspergillus flavus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Paecilomyces* and *Penicillium* on historic stone buildings in Serbia. De la Torre and coworkers (De la Torre et al., 1993; De la Torre & Gómez, 1994) have highlighted the acidogenic capacity of *Alternaria tenuissima* (isolated from wood), *Cladosporium* (from paper, textiles and wood), *Mucor* and *Trichoderma* (from textiles) and *Fusarium* (from protein-containing materials).

A total of 46% of *Aspergillus* strains and 73% of *Penicillium* strains displayed capacity to produce diffusible pigments, thus confirming their potential as biodeterioration agents. Colour was maintained regardless of substrate: *Aspergillus terreus* strains isolated from paper and textiles produced amber pigments in both cases, while *Penicillium chrysogenum* strains (isolated from paper and from lignocellulose-containing, protein-containing and inorganic substrates) were always yellow. Hua et al. (1999) report that mutant *Aspergillus flavus* strains accumulate large amounts of norsolorinic acid on Czapek medium, giving rise to a reddish-orange pigment; the same hue was produced by the *A. flavus* strain isolated from textiles in the present study; the yellow pigment detected in *A. niger* has also been reported by Rosas and Casadevall (1997). Pigments observed for *Penicillium* were mostly yellow (*P. citrinum* and *P. glabrum*), although *P. purpurogenum* displayed the reddish pigment characteristic of this species (Pitt, 2000).

Ability to degrade protein-based components such as gelatine and casein was found for most *Aspergillus* strains, for *Penicillium chrysogenum* and *P. glabrum*, and for *Cladosporium oxysporum*. Proteinaceous glues, including animal gelatines, albumin, casein and egg, have been used for centuries as binding media in paintings to cover and bind pigments and to protect

the outer layer of finished pictures (Peris-Vicente et al., 2005). Casein is also common in the canvas cloth used as reinforcement on the back of frescoes (Colombini et al., 1999; Ranalli et al., 2005).

The results showed the high potentialities of the studied fungi for the biodeterioration of stored materials. Also, as reported by other authors, when relative humidity rises above 70%, the risk for the apparition of fungi capable of colonising the susceptible substrates increases (Agrawal & Dhawan, 1985). However, Shelton et al. (2002) suggested that high relative humidity coupled with deficient ventilation of indoor locations promotes the occurrence of fungal contamination that could lead to biodeterioration.

Conclusion

The fungal species isolated from stored heritage objects are the cause of biodeterioration, which, in some cases, is even visible to the naked eye. Moreover, these fungi pose a potential threat for as-yet-undamaged items in the same collections. In this study, characterisation of airborne fungi and of species isolated from stored material, followed by evaluation of their biodeterioration capacity, provided the basis for a set of recommendations to the institutions concerned regarding the implementation of proper protection measures. Prophylactic and preventive measures should be applied, taking in consideration each location. Among preventive measures, the inspection and frequent cleaning of the stored heritage objects, as well as adequate ventilation and the prevention of the entrance of air from outside, could contribute to the decrease of relative humidity and consequently, fungal contamination, in order to prevent their establishment on the substrate surfaces.

Species investigated

Aspergillus caespitosus Raper et Thom
Aspergillus candidus Link
Aspergillus clavatus Desm.
Aspergillus flavus Link
Aspergillus fumigatus Fresen.
Aspergillus niger Tiegh.
Aspergillus oryzae (Ahlburg) E. Cohn
Aspergillus sydowii (Bainier et Sartory) Thom et Church
Aspergillus tamaritii Kita
Aspergillus terreus Thom
Aspergillus versicolor (Vuill.) Tirab.
Alternaria alternata (Fr.) Keissl.
Chaetomium bostrychodes Zopf
Chaetomium globosum Kunze
Chaetomium indicum Corda
Cladosporium cladosporioides (Fresen.) G.A. de Vries
Cladosporium herbarum (Pers.) Link.
Cladosporium oxysporum Berk. et M.A. Curtis
Cladosporium sphaerospermum Penz.
Curvularia lunata (Wakker) Boedijn

Curvularia pallescens Boedijn
Chrysonilia sitophila (Mont.) Arx
Emericella nidulans (Eidam) Vuill.
Eurotium chevalieri L. Mangin
Fusarium oxysporum Schldt.
Lentinus hirtus (Fr.) Murrill
Mucor circinelloides Tiegh.
Nigrospora sphaerica (Sacc.) E.W. Mason
Penicillium chrysogenum Thom
Penicillium citrinum Thom
Penicillium corylophilum Dierckx
Penicillium glabrum (Wehmer) Westling
Penicillium janthinellum Biourge
Penicillium purpurogenum Stoll
Penicillium simplicissimum (Oudem.) Thom
Paecilomyces variotii Bainier
Rhizopus oryzae Went et Prins. Geerl.
Rhizopus stolonifer (Ehrenb.) Vuill.
Syncephalastrum racemosum Cohn ex J. Schröt.

References

- Abin, L., Coto, O., Gómez, Y., Marrero, B. & Marrero, J. (2002). Effect of different factors on citric acid production by *Aspergillus niger* O5. *Revista del Centro Nacional de Investigaciones Científicas. Ciencias Biológicas*, 33, 15–18.
- Abrusci, C., Martín-González, A., Del Amo, A., Catalina, F., Collado, J. & Platas, G. (2005). Isolation and identification of bacteria and fungi from cinematographic films. *International Biodeterioration and Biodegradation*, 56, 58–68.
- Agrawal, O. P. & Dhawan, S. (1985). *Control of biodeterioration in museums. Technical note 2*. Lucknow: National Research Laboratory for Conservation of Cultural Property.
- Aira, M. J., Rojas, T. I. & Jato, V. (2002). Fungi associated with three houses in Havana (Cuba). *Grana*, 41, 114–118.
- Aira, M. J., Jato, V., Stchigel, A. M., Rodríguez-Rajo, F. J. & Piontelli, E. (2007). Aeromycological study in the Cathedral of Santiago de Compostela (Spain). *International Biodeterioration and Biodegradation*, 60, 231–237.
- Atlas, R. M. (2000). *Handbook of microbiological media*. Boca Raton, FL: CRC Press.
- Borrego, S., Pons, V. & Perdomo, I. (2008). La contaminación microbiana del aire en dos depósitos del Archivo Nacional de la República de Cuba. *Revista del Centro Nacional de Investigaciones Científicas. Ciencias Biológicas*, 39, 63–69.
- Borrego, S., Guamet, P., Gómez de Saravia, S., Batistini, P., García, M., Lavin, P. & Perdomo, I. (2010). The quality of air at archives and the biodeterioration of photographs. *International Biodeterioration and Biodegradation*, 64, 139–145.
- Burch, M. & Levetin, E. (2002). Effects of meteorological conditions on spore plumes. *International Journal of Biometeorology*, 46, 107–117.
- Colombini, M. P., Modugno, F. & Giacomelli, A. (1999). Two procedures for suppressing interference from inorganic pigments in the analysis by gas chromatography/mass spectrometry of proteinaceous binders in paintings. *Journal of Chromatography*, 846, 101–111.
- Das, M. K. L., Prasad, J. S. & Ahmad, S. K. (1997). Endoglucanase production by paper-degrading mycoflora. *Letters in Applied Microbiology*, 25, 313–315.
- De la Torre, M. A., Gómez, G., Viscaino, C. & García, T. M. (1993). Biochemical mechanisms of stone alteration carried out by filamentous fungi living in monuments. *Biogeochemistry*, 19, 129–147.
- De la Torre, M. A. & Gómez, G. (1994). Manganese and iron oxidation by fungi isolation from building. *Microbial Ecology*, 27, 117–180.

- Desai, A. J. & Betrabet, S. M. (1972). Cellulase activity of microorganisms isolated from cotton deteriorated during storage. *Indian Journal of Biochemistry and Biophysics*, 9, 34–39.
- Federación Española de Empresas de Calidad Ambiental Interior (FEDECAI). (2007). *Programa de certificación de calidad ambiental en interiores. Calidad ambiental en interiores: Criterios de muestreo*. Madrid: FEDECAI.
- Florian, M. L. (2003). Water, heritage photographic materials and fungi. *Topics in Photographic Preservation*, 10, 60–73.
- Herrera, A. (1985). *Manual de medios de cultivo*. La Habana: Editorial Científico-Técnica.
- Hua, T., Baker, J. L. & Flores, E. (1999). Melanin interactions of saprophytic yeasts with a nor mutant of *Aspergillus flavus*. *Applied and Environmental Microbiology*, 65, 2738–2740.
- Hyvärinen, A., Vahteristo, M., Meklin, T., Jantunen, M. & Nevalainen, A. (2001). Temporal and spatial variation of fungal concentrations in indoor air. *Aerosol Science and Technology*, 35, 688–695.
- Inter-Society Color Council-National Bureau of Standards (ISCC-NBS). (1964). *Methods of designating color and dictionary of color names. Supplement to NBC circular 553*. Washington, DC: United States Department of Commerce.
- Kasznica-Kocot, J., Lis, D. O., Kordys-Darmolińska, B., Grzybowska-Chlebowczyk, U., Wos, H. & Górny, R. L. (2007). Children's allergic diseases and microbiological contamination of indoor air. A case report. *Annals of Agricultural and Environmental Medicine*, 14, 187–190.
- Klich, M. A. & Pitt, J. I. (1994). *A laboratory guide to the common Aspergillus species and their teleomorphs*. North Ryde: CSIRO Division of Food Research.
- Ljaljevic, M. V. & Vukojevic, J. B. (2009). Role of fungi in biodeterioration process of stone in historic buildings. *Proceedings for Natural Sciences*, 116, 245–251.
- Lugauskas, A., Levinskaite, L. & Peculyte, D. (2003). Micromycetes as deterioration agents of polymeric material. *International Biodeterioration and Biodegradation*, 52, 233–242.
- Nugari, M. P. & Roccardi, A. (2001). Aerobiological investigations applied to the conservation of cultural heritage. *Aerobiologia*, 17, 215–223.
- Ovando-Chacon, S. L. & Waliszewski, K. N. (2005). Preparativos de celulasas comerciales y aplicaciones en procesos extractivos. *Universidad y Ciencia*, 21, 111–120.
- Peltola, J., Andersson, M., Rainey, F. A., Haahtela, T., Mussalo-Rauharnaa, H., Samson, R. & Salkinoja-Salonen, M. S. (2001). Toxic metabolite producing bacteria and fungi in indoor environment. *Applied and Environmental Microbiology*, 67, 3269–3274.
- Peris-Vicente, J., Simó-Alfonso, E., Gimeno Adelantado, J. V. & Doménech Carbó, M. T. (2005). Direct infusion mass spectrometry as a fingerprint of protein-binding media used in works of art. *Rapid Communications in Mass Spectrometry*, 19, 3463–3467.
- Pitt, J. I. (2000). *A laboratory guide to common Penicillium species*. Sydney: Food Science Australia.
- Pointing, S. B. (1999). Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. *Fungal Diversity*, 2, 17–33.
- Portugal, N. M. A., Videira, S., Ecchevarria, S. R., Bandeira, A. M. L., Santos, M. J. A. & Freitas, H. (2009). Fungal diversity in ancient documents. A case study on the archive of the University of Coimbra. *International Biodeterioration and Biodegradation*, 63, 626–629.
- Ranalli, G., Alfano, G., Belli, C., Lustrato, G., Colombini, M., Bonaduce, I., Zanardini, E., Abbruscato, P., Cappitelli, F. & Sorlini, C. (2005). Biotechnology applied to cultural heritage: Biorestitution of frescoes using viable bacterial cells and enzymes. *Journal of Applied Microbiology*, 98, 73–83.
- Ranalli, G., Zanardini, E. & Sorlini, C. (2009). Biodeterioration – including cultural heritage. In M. Schaechter (Ed.), *Encyclopedia of microbiology* (pp. 191–205). Amsterdam: Elsevier.
- Robledo, M. & Moretti, C. (1986). Aislamientos de algunos hongos que atacan documentos en el Archivo General de la Nación. *Revista Mexicana de Micología*, 2, 125–130.
- Rojas T. I., Martínez, E., Gómez, Y. & Alvarado, Y. (2002). Airborne spore of *Aspergillus* in cultural institutions at Havana University. *Grana*, 41, 190–193.
- Rojas, T. I., Martínez, E., Aira, M. J. & Almaguer, M. (2008). Aeromicrota de ambientes internos: Comparación de métodos de muestreo. *Boletín Micológico*, 23, 67–73.
- Rosas, A. L. & Casadevall, A. (1997). Melanization effects susceptibility of *Cryptococcus neoformans* to heat and cold. *Microbiology Letters*, 153, 265–272.
- Salkinoja-Salonen, M., Peltola, J. & Andersson, M. A. (2003). Microbial toxins in moisture damaged indoor environment and cultural assets. In C. Sainz-Jimenez (Ed.), *Molecular biology & cultural heritage* (pp. 93–108). Lisse: Balkema.
- Sánchez, A. I., Martínez, P., Borrego, S. & Brizuela, A. L. (1988). Estudio de la contaminación fúngica en vigas de madera de la iglesia del Espíritu Santo. *Revista Cubana de Biología*, 11, 16–33.
- Shelton, B. G., Kirkland, K. H., Flanders, W. D. & Morris, G. K. (2002). Profiles of airborne fungi in buildings and outdoor environments in the United States. *Applied and Environmental Microbiology*, 68, 1743–1753.
- Stryjowska-Sekulska, M., Piotraszwska-Pająk, A. & Filipiak, M. (2005). Outdoor and indoor air fungal microflora of academic buildings in Poznań. *Abstracts of AEROTOP Fungal Workshop, Poznan, Poland, 8–10 April 2005*, 34–43.
- Thakre, R. P., Bhajbhujje, M. N. (1991). Biodeterioration of books and journals. *Proceedings of the International Conference on Biodeterioration of Cultural Property, New Delhi, India, 20–25 February 1989*, 139–147.
- Vaillant, M., Chi, L. & Sánchez, A. I. (1989). Sobre la contaminación microbiológica existente en depósitos del Archivo Nacional. *Documentos*, 2, 44–65.
- Valentín, N. (2003). Microbial contamination in museum collections: Organic materials. In C. Sainz-Jimenez (Ed.), *Molecular biology & cultural heritage* (pp. 85–92). Lisse: Balkema.
- Villalba, L. S., Mikán, J. F. & Sánchez, J. (2004). Actividades hidrolíticas y caracterización isoenzimáticas de poblaciones microbianas aisladas del patrimonio documental del Archivo General de Colombia. *Nova Publicación Científica*, 2, 50–57.