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GILDED WOODCARVING ALTERATION: ASSESSMENT OF FILAMENTOUS FUNGI ACTION

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Abstract

Biodegradation and biodeterioration by living organisms can cause massive damage to historical monuments. Fungi are a major responsible in wood degradation sharing a single strategy for degrading wood polymers by secreting enzymes that break down the main constituents of wood such as cellulose or xylose. In this work, the presence and participation of fungi in woodcarving and their biological activity were examined by monitoring their cellulolytic/xylanolytic activity. Isolated fungi of the genera Penicillium, Cladosporium and Mucor showed the highest cellulose and xylose activity and are therefore the main responsible for the structural deterioration of wood support of the altars of the Espírito Santo Church, Évora, Portugal. The application of combined strategies allow a fast and efficient screening to signalise the main biodegradative agents in gilded woodcarving.

Keywords: *Biodegradation; Gilded woodcarving; Cellulolytic/xylanolytic activity; Wood decay; Fungal development*

Introduction

Wood has been used since Antiquity as one of the major materials for construction and production of objects and artworks.

This natural material consists of an orderly arrangement of cells with walls composed by different amounts of three biopolymers like cellulose, hemicellulose and lignin. There is a great diversity of woody plants enabling different types of wood reflected by distinct morphology and chemical composition [1, 2]. Their decay is important for ecosystem functioning and recycling of organic matter in the environment, but sometimes this natural process leads to destruction of wooden objects of historic and cultural value [3]. One of the main purpose when dealing with conservation of these artworks is to understand what type of deterioration occurs and how these processes impact the wood degradation [1].

Wood, as biodegradable natural material, is permanently exposed to degradation phenomena promoted by environmental, chemical and/or microbial nature. The extent of the deterioration depends on the environment in which the material is inserted. There are several environmental conditions that contribute significantly to the degradation of wood such as humidity, temperature, solar irradiation time, atmospheric ozone content and pollution. The

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main chain and/or the side chains may be affected by the degradation process and, consequently, irreversible losses of material occur [4, 5].

Particularly relevant for this issue are the biodegradation process, where insects, fungi and bacteria can act synergistically in the wood decay. Between the different agents associated with this phenomenon, fungi have a particular impact in the wood decay, having the ability to produce extracellular enzymes that break down the woody cell wall [1, 6].

Thus, fungal communities, due to their saprophyte nutritional mode, contribute significantly not only to the degradation of wood in nature but also to the deterioration of wooden objects of cultural importance, causing aesthetic and structural damage [3].

Fungi taxonomically classified in the subdivision Basidiomycota (white- and brown- rot fungi) and Ascomycota (soft rot fungi) have been implicated in the wood alterations, having the first one a crucial role in this process [7]. White rot fungi can degrade all cell wall components, including lignin. They often cause a bleaching of normal wood coloration. Their ability to metabolise large amounts of lignin in wood is unique among microorganisms. Brown-rot fungi depolymerase cellulose rapidly during incipient stages of wood colonisation. Considerable losses in wood strength occur very early in the decay process, often before decay characteristics are visually evident [1, 8]. Soft-rot decay is characterised by cavities formed within the cell walls of wood following the microfibrillar orientation of cellulose. This type of decay occurs generally when wood is exposed to excessive moisture, but it can also occur in dry environments. In this way, the growth of the microorganism in wood and the type of degradative system produced results in different decay patterns being produced [3].

On the other hand, the pH of the wood is also an important factor that could have an effect on the type of fungi that may become established. Thus, depending on the type of decay, different physical, chemical and morphological changes can occur in wood [1].

This biodegradable support has been widely used in gilded wood production, particularly in religious monuments but also in non-religious decorations like libraries and palaces [9].

Gold, considered the most precious of the metals with properties that seemed to remain unaltered throughout time, was massively used during the 17th and 18th century, for gilding woodcarving monumental altarpieces, side chapels, statues and decorative wood panels, thus creating an image of eternity and luxuriousness in the artworks [10]. Unfortunately, in many cases these artistic representations have suffered alterations over time whose main players seem to be the biological agents, which have ability to degrade the wood support.

Bearing this in mind, gilded wood alterations of two different altars (Fig. 1) present in the *Espírito Santo* Church - *Santa Ursula* Altar/ *Nossa Senhora da Boa Morte* Altar (Évora, Portugal) - were explored in this study in order to characterize the agents that promote degradation of this form of art.

Fig. 1. Schematic representation of the sampling process performed in the *Santa Ursula* (A) and *Nossa Senhora da Boa Morte* (B) Altars, in the *Espírito Santo* Church (Évora, Portugal).

Materials and Methods

Sampling

Biological materials present in gilded woodcarving from the two altars of *Espírito Santo* Church (Évora, Portugal) were aseptically collected in microtubes sterile scalpels and microtubes. The selection of these altars took into consideration the visual alterations of the support. Figure 1 illustrates the sampling locations for material and biological identification and characterization.

In vitro simulation of woodcarving colonisations

The collected biological samples were diluted in sterile MRD medium (Maximum Recovery Diluent, Merck) and shaken mechanically for 1 h under aseptic conditions. Bacterial isolation procedures were carried out in Petri dishes containing NA (Nutrient Agar) at 30ºC, for 48 h. The distinct obtained single colonies were sub-cultured onto NA for characterization. Bacterial strains were maintained on NA slants at 4ºC.

Fungal isolation of the colonies was performed successively, using standard mycological medium (Malt Extract Agar- MEA and Cook Rose Bengal- CRB). All cultures were grown for 7 days at 28ºC. Identification of fungi was based on the macroscopic features of colonies grown on agar plates, and the micro-morphology of the reproductive structures was identified by optical microscopy (OM). Fungal strains were identified following standard methods [11], based on their macro and micro-morphological characteristics.

Biological contamination assessment

The elemental compositions were examined by variable pressure scanning electron microscopy coupled with energy dispersive X-ray spectrometry (VP-SEM-EDS) using an Hitachi Scanning Electron Microscope S-3700N (Tokyo, Japan) with the accelerating voltage 18–20 kV coupled to a Bruker XFlash 5010 energy dispersive X-ray spectrometer (Berlin, Germany). This analysis was performed in order to detect microbial contamination and the elemental composition of the materials analyzed (point analysis and two-dimensional mapping). In order to assess the degree of deterioration of the support and the type of colonising microorganisms, samples were coated with Au-Pd (Balzers Union SCD 030) during 30 s, and observed with the same microscope with an accelerating voltage of 10-15 kV.

Biodegradative enzymes activity

To allow a deeper insight into the woodcarving deterioration role of the isolated fungi, cellulolytic and xylanolytic activity produced by fungi was determined according to Heck et al. 2002. Cellulose is an unbranced glucose polymer composed of anhydro-D-glucose units linked by 1,4-β-D-glucoside bonds, which can be hydrolysed by cellulolytic enzymes produced by fungi. Xylan has a linear backbone comprised of b-1,4-linked D-xylopyranose residues, which, depending on the origin, may present ramifications containing mainly acetyl, arabinosyl and gluconosyl residues [12]. The fungi *Trichoderma harzianum* CCMI 783 (F8) and *Trichoderma koningii* CCMI 868 (F9) were used as positive control.

Results and Discussion

The first approach consisted in observing the gilded woodcarving microsamples collected in the altars by VP-SEM-EDS to allow the chemical composition of the gold leaf (data not shown) and the signalisation of the biological contamination (Fig. 2).

VP-SEM-EDS analysis evidenced the presence of characteristic elements of biological agents present in *Santa Ursula* Altar and *N. S. da Boa Morte* Altar. The combination of carbon, oxygen, sulphur and nitrogen in the structure observed confirms the microbial contamination in the gilded wood.

On the other hand, SEM micrographs on coated samples corroborate the evidences obtained by VP-SEM-EDS, clearly showing microbiological proliferation (Fig. 3) on the altars.

It is possible to observe fungal spores dispersion and filamentous fungi proliferation, whose hyphae wood penetration promotes the disintegration of the matrix and the wood fibres.

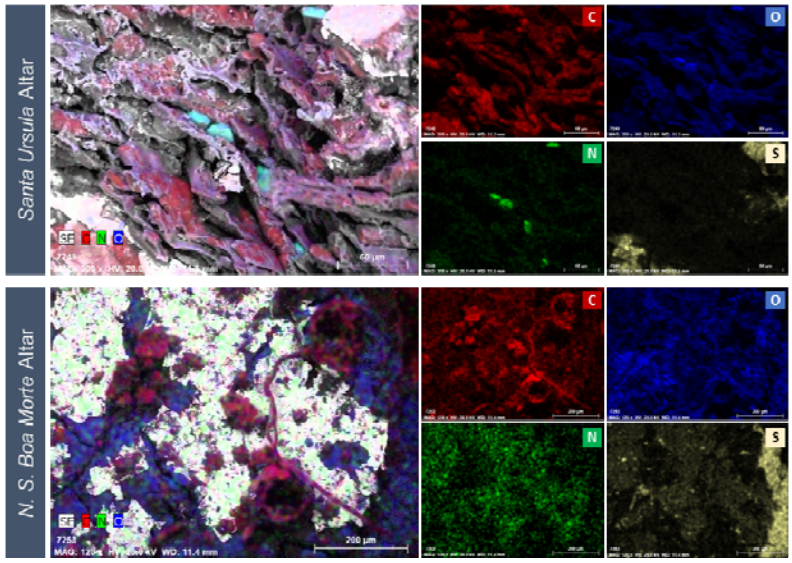


Fig. 2. SEM micrograph and EDS 2D mapping of gilded woodcarving microfragments from the *Santa Úrsula* and *N. S. da Boa Morte* Altars, with elemental maps of carbon (C), oxygen (O), nitrogen (N) and sulphur (S).

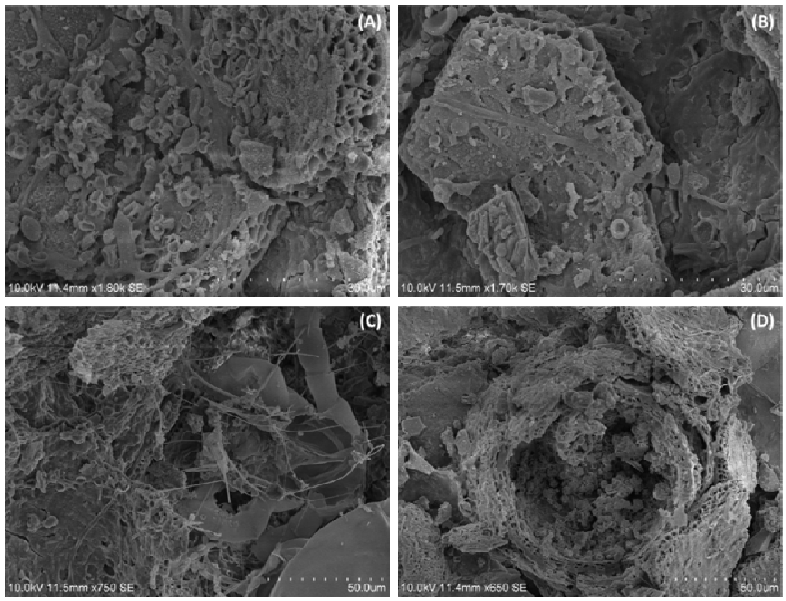
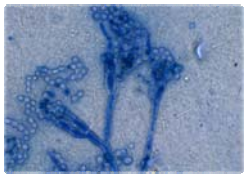
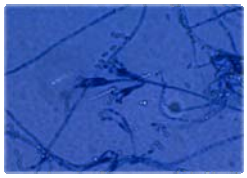
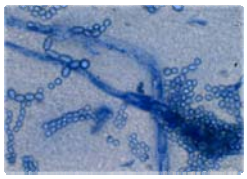
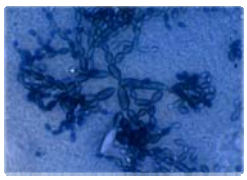
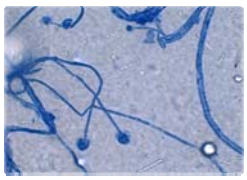
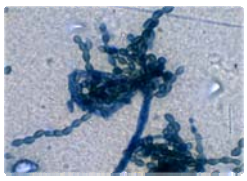
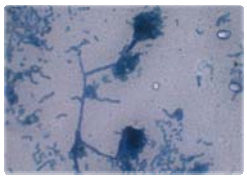


Fig. 3. SEM micrographs obtained by the analysis of microfragments from the altars of *Espírito Santo* Church, evidencing biological proliferation of fungi.

To complement this information, culture-dependent methods were used, enabling the knowledge of the cultivable microorganisms. The population present in the altars is mainly composed by filamentous fungi and some bacteria. The fungal community identified was quite diversified (Table 1): 4 different strains of *Penicillium* sp., 2 strains of *Cladosporium* sp. and one strain of *Mucor* sp..

Table 1. Macroscopic and microscopic features of the fungal population isolated from *Espírito Santo* Church.

Code	Macroscopic features	Microscopic features	Identification
F1			*Penicillium* sp.1
F2			*Penicillium* sp.2
F3			*Penicillium* sp.3
F4			*Cladosporium* sp.1
F5			*Mucor* sp.1
F6			*Cladosporium* sp.2
F7			*Penicillium* sp.4

This fungal population was found in areas with high alteration levels, particularly with wood fibres disintegration/disorganisation as was observed by SEM and compared with non-altered wood (Fig. 4). According to this, the biodegradative capacity of each isolated fungus was investigated by the monitoring of cellulase and xylanase enzymatic activity, since these enzymes are involved in the wood degradation process [13].

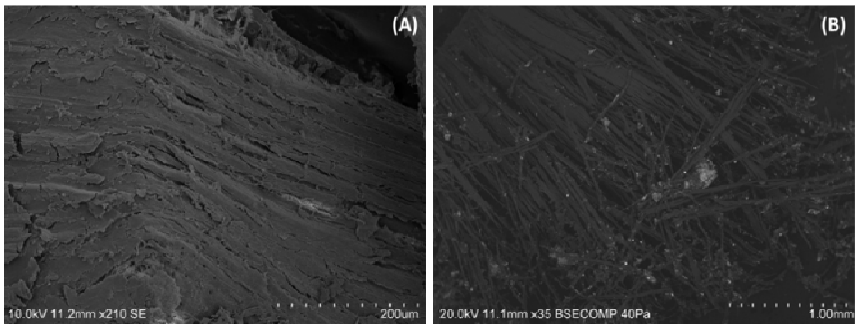


Fig. 4. Morphological aspect of non-altered wood (A) and degraded wood (B) from *Espírito Santo* Church, observed by SEM imaging.

The results obtained show that all the fungi isolated from gilded wood produce cellulolytic enzymes (Fig. 5). However each microorganism has different enzymatic profiles during the incubation period, as shown in figure 5. The fungi F4 and F5 (*Cladosporium* sp.1 and *Mucor* sp.1) have the highest cellulolytic activity at 72 h of incubation, while fungi F1, F3 and F7 (*Penicillium* sp.1, *Penicillium* sp. 3 and *Penicillium* sp.4) show a maximum activity at 340 h. On the other hand, the fungi F6 and F2 (Cladosporium sp.2 and Penicillium sp.2) reveal the highest enzyme activity at 24h and 48h, respectively.

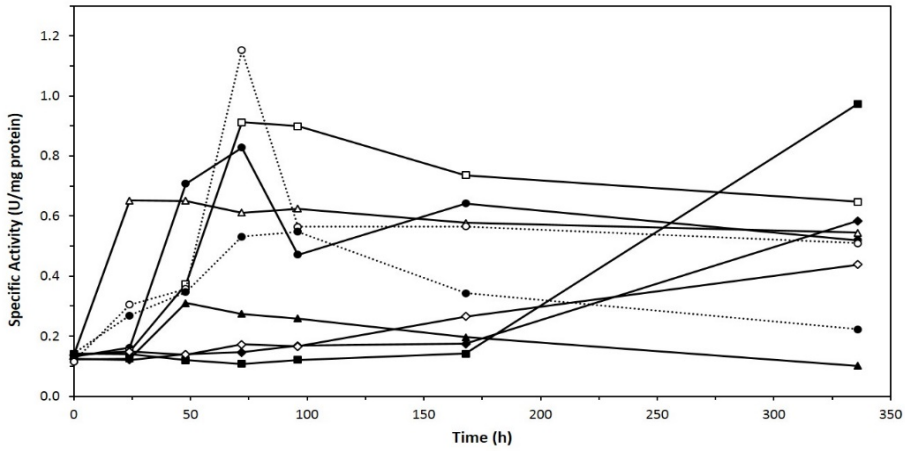


Fig. 5. Cellulolytic activity of the fungal population isolated from *Espírito Santo* Church
(F1; F2; F3; F4; F5; F6; F7; F8; F9.

In these assays, two fungi known to produce these enzymes - *Trichoderma harzianum* CCMI 783 (F8) and *Trichoderma koningii* CCMI 868 (F9) –, were used as control, making a comparison possible between the enzymatic levels of the tested microorganisms. In this way, the fungi F5 (*Mucor* sp. 1) and F4 (*Cladosporium* sp.1) have enzymatic levels very similar to *Trichoderma harzianum* CCMI 783.

According to these results, cellulase enzyme activities vary widely among the fungal strains present in the wood, which potentiates a synergetic deteriorative effect [14].

Xylanolytic activity and wood-degrading ability by different fungi mostly wood-inhabiting, was also investigated.

This enzyme has a similar behaviour to the cellulase activity. However the highest enzymatic levels are obtained at different times (Fig. 6). The fungi F5, F6 and F8 reach the maximum activity at 72 h, while F4 has two different enzymatic maximum activities (48 h and 168 h). On the other hand, the fungi F1, F2, F3 and F7 have low enzymatic activities.

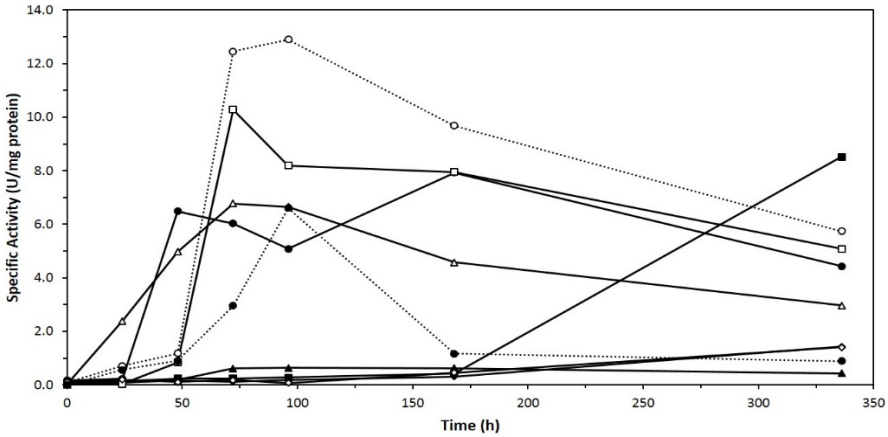


Fig. 6. Xylanolytic activity of the fungal population isolated from *Espírito Santo* church (F1; F2; F3; F4; F5; F6; F7; F8; F9.

Besides that, the enzymatic activity of xylanases was higher than cellulases, probably because structures of hemicelluloses are easily hydrolysable polymers than cellulose. Furthermore, some studies reported that although xylan has higher complexity in comparison with cellulose since this polymer does not form tightly packed structures, which results in an easier accessibility to hydrolytic enzymes [15].

Therefore, this study indicates that the presence of several biological communities can promote a widespread degradation, since each microorganism has distinct enzymatic profiles and different ways to act. These indicators show the important contribution of the microorganisms for the biodegradation process, whose action needs to be stopped in order to prevent the loss of the artworks under study.

Conclusions

The predominant microbial agents detected in the gilded woodcarving were filamentous fungi of the genera *Penicillium*, *Cladosporium* and *Mucor* that seem to be the main agents for the visual alteration and structural deterioration of wood support of the studied altars. Their degradative potential were inferred by the presence of cellulolytic and xylanolytic enzymes that have the ability to degrade the wood fibres.

The application of combined strategies allowed a fast and efficient screening to signalise the main biodegradative agents in gilded woodcarving.

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