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ORIGINAL ARTICLE

Heterotrophic components of biofilms on wood artefacts

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Abstract

Heterotrophic components of biofilms on wood artefacts were studied at the Conservation Laboratory for Wood Artefacts of the University Suor Orsola Benincasa of Naples, Italy. The aim of the study was to add new information on the microhabitats represented by biofilms formed by wood-dwelling organisms. Light and electron microscopy of histological features of woods used to make the artefacts showed that the woods belonged to species of lime (*Tilia* sp.), poplar (*Populus* sp.) and pear (*Pyrus* sp.). A Denaturing Gradient Gel Electrophoresis analysis performed on heterotrophic microorganisms colonizing the artefacts led to identify four species of bacteria, namely *Bacillus cereus, B. mycoides, B. subtilis* and *Microbacterium oleivorans*, and seven species of fungi, namely *Alternaria alternata, Aspergillus fumigans, A. versicolor, Cladosporium cladosporioides, C. oxysporum, Fusarium oxysporum* and *Penicillium chrysogenum*. Based on its morphological features, an insect found on some artefacts was identified as the xylophagous beetle *Nicobium castaneum* (Anobiidae). The influence of wood type and environmental conditions on the diversity of microorganisms was discussed.

Keywords Bacteria · Biofilm · Fungi · Insects · Wood

Introduction

Artefacts are continuously exposed to the effects of physical, chemical and biological factors which facilitate the growth of a wide variety of colonizing microorganisms, such as bacteria, cyanobacteria, algae, filamentous fungi and lichens, as well as of higher organisms, such as mosses, weeds and insects [1, 2].

The type of substrate also affects the diversity of the microbial communities, with quantity and quality of organisms varying according to whether the organisms grow on stone materials [1, 3–6] or on organic materials, such as woods and painted tissues [1]. The interactions between the organisms and the substrates, together with abiotic factors (e.g., wind, rain, humidity and pollution), cause physical, chemical and aesthetical damages to artefacts [1, 3–6].

In indoor environments, as museums and exhibition halls, where the light is limited or controlled, autotrophic microorganisms are absent or rare, whereas fungi and bacteria easily grow, playing the most important role in biodeterioration processes of the artefacts; insects contribute in determining such processes [1, 7–9].

Particularly exposed to microbial attack in indoor environments are wood artefacts. Any deteriogenic organism finds on the wood an elective substrate for its settlement, due to porous wood structure, and diversified sources of nutrients for its growth, due to variety of organic matters supplied by the wood cellular components [10–15].

Microbial wood infections are mostly airborne and a high number of fungal and bacterial spores can accumulate in dust layers [16]. Virtually no wood exists that cannot be damaged by fungi [1]. Fungi reproduce and disperse through spores, which, after a period of quiescence, or dormancy, germinate, in some cases only after an activation phase [17, 18]. Some chemicals, such as detergents, organic acids, alcohols and other solvents usually employed in restoration, may act as activators [14, 19]. An equally important role in the wood biodeterioration process is played by bacteria [12], especially by those

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that foster colonization of other microorganisms with the products of their metabolites [12, 20]. The initial colonization of the wood by bacteria increases the permeability of the wood structure through the penetration of bacteria in the cavities of the cell walls, so preparing the fungal attack [21]. Other bacteria, as the cellulolytic ones, have the ability to degrade lignin and other components of the wood, resins, gums, dyes, tannic acid, waxes and fats [22]. In addition, amylolytic bacteria, as *Bacillus* and *Clostridium*, are capable of degrading the starch and lipolytic bacteria, e.g., *Bacillus, Alcaligenes, Staphylococcus* and *Clostridium*, are capable of degrading lipids thanks to the production of lipase [22, 23]; the latter microorganisms contribute in the deterioration of artefacts containing fatty substances as natural components of the wood [12, 21, 24]. Insects are also involved in determining structural alterations in the wood artefacts for their biodegradative activity [25, 26].

Several works are available which describe bacterial and autotrophic communities involved in the degradation of art objects [4–6, 15, 27], whereas comparatively fewer investigations focus on the heterotrophic microbial communities and insects responsible for the biodeterioration of wood artefacts.

In the present study, we investigated heterotrophic communities dwelling on wood artefacts, identified the type of wood used to carve the artefacts and verified the occurrence of insects on the woods. We also took into account the effects on the wood deterioration by environmental factors in the rooms hosting the artefacts.

Materials and methods

Artefacts investigated

Polychrome wood artefacts were investigated. Samples for the analyses were collected from the artefacts at the Conservation Laboratory for Wood Artefacts of the University Suor Orsola Benincasa of Naples, Italy, where the artefacts were temporary transferred to undergo restoration interventions. A description of the investigated artefacts follows.

Immacolata Concezione con Bambino (Immaculate Conception with Child) (hereinafter referred to as Artefact 1) (Fig. 1a). It is a sculpture exposed in a niche in the *Sala degli Angeli* (Hall of the Angels) at the University Suor Orsola Benincasa of Naples, Italy [28]. The sculpture dates back to the mid-eighteenth century [29, 30]. The sculpture was subject to a significant biological attack by bacteria and fungi as well as by wood infesting insects [28].

Bambinello (Baby Jesus) (hereinafter referred to as Artefact 2) (Fig. 1b). It is a sculpture belonging to a private collection, dating back to nineteenth century. The sculpture presented an advanced state of biodeterioration mainly due to microbial attack.

Croce Girolamini (Girolamini Cross) (hereinafter referred to as Artefact 3) (Fig. 1c). It is a sculpture exposed at the Girolamini Museum of Naples, dating back to the fourteenth century [31]. Diffuse attacks by biodeteriogens were present on the sculpture.

Painting frames (hereinafter referred to as Artefact 4) (Fig. 1d). They are from ten paintings stored at the University Suor Orsola Benincasa of Naples. Their historical

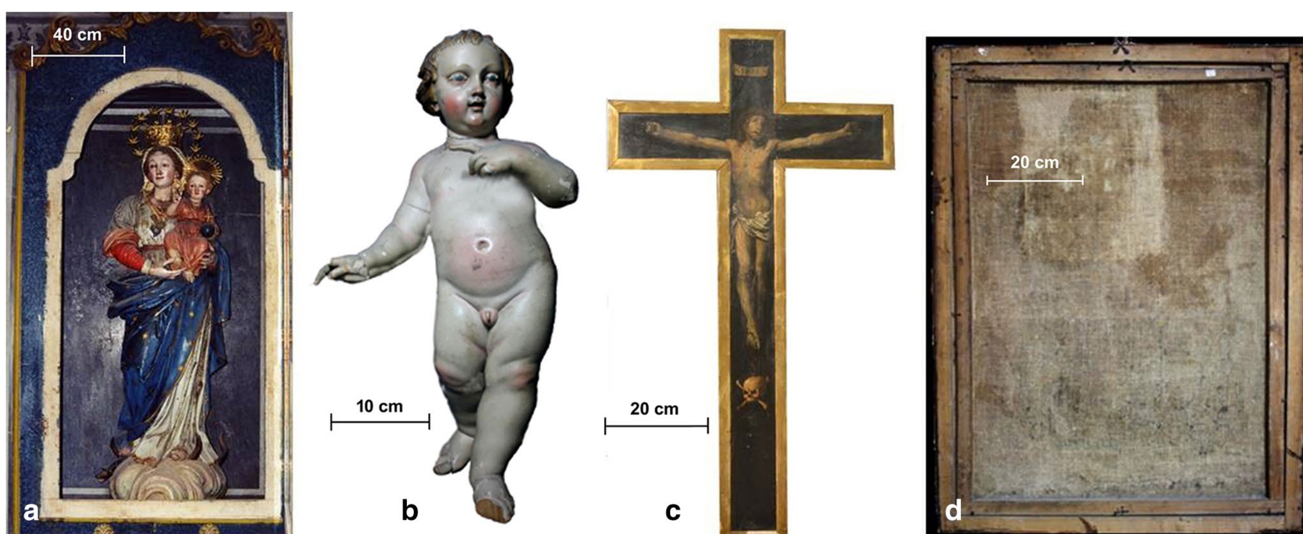

Fig. 1 Artefacts investigated. **a** Artefact 1, **b** Artefact 2, **c** Artefact 3, **d** Artefact 4

production period is from seventeenth to nineteenth century [32]. The frames showed microbial and insects attack.

Environmental factors

Light intensity, air temperature and relative humidity values recorded over the years 2011–2016 in the rooms hosting the artefacts are summarized in Table 1. They were provided by the Conservation Laboratory for Wood Artefacts of the University Suor Orsola Benincasa of Naples, Italy. Light intensity was measured at noon. Air temperature and relative humidity were recorded at intervals of 15 min.

Identification of woods

Micro-samples were collected from parts of woods not covered by paints or that were exposed due to fractures or removal of the paints for restoration works.

Light microscopy and scanning electron microscopy (SEM) observations of wood section features were performed for the identification of the woods. For interpretation of images, we referred to analytical keys texts [33, 34]. Terminology according to Schweingruber [34] was adopted for descriptions of characters useful for wood determination.

For light microscopy, transverse and/or longitudinal (tangential and radial) handmade sections of sampled woods were observed with a Zeiss Axiolab microscope (Zeiss, Jena, Germany) and photographed with a Nikon Digital Sight DS-L1 (Nikon, Tokyo, Japan). For SEM observations, wood samples were coated with gold to about 30 nm. The samples were observed under a FEI Quantas 200 (FEI Company, Hillsboro, USA) environmental scanning electron microscope at an accelerating voltage of 25 kV. SEM observations were carried out at the Laboratory of Measures in Electron and Confocal Microscopy of the Centre of Advanced Metrological Services of the University of Naples Federico II, Naples, Italy.

Table 1 Averages of minimum and maximum values of temperature (*T*), light intensity (LI) and relative humidity (RH) reported for the rooms hosting the artefacts over the years 2011–2016

	T (°C)	LI (Lux)	RH (%)
Artefact 1	17–20	79–83	58–68
Artefact 2	17–20	80–120	55–60
Artefact 3	24–28	65–93	65–75
Artefact 4	24–30	73–80	60–88

Standard deviations of all measured parameters values resulted within ±2

Identification of insects

For the entomological identification, the morphology and size of insects, exit holes and powdered frass were observed by an optical microscope in reflected light (Nikon SMZ 1500, Nikon, Tokyo, Japan) and photographed with a Nikon Digital-Sight DS-Fi1 (Nikon, Tokyo, Japan). The diameter of exit holes was measured by using a caliper.

Sampling and cultivation of microorganisms

Samples were collected at different times for each artefact, from 2011 to 2016, depending on the periods in which each artefact was subjected to restoration work. Samples of Artefacts 1 and 3 were collected in 2011, samples of Artefact 2 in 2016, while the samples of Artefact 4 (consisting of various wooden frames) were collected from 2011 to 2016.

Six samples were collected from each artefact. For Artefact 1, samplings were performed on wood devoid of paint on the back of the sculpture; for the Artefact 2, on a finger of the hand and on a foot where wood was exposed for fractures; for the Artefact 3, on a not painted area on its back; and for the Artefact 4, from different areas of the frames after removal of paintings (Fig. 1).

For collecting and cultivation procedures, we followed the protocol reported by Milanesi et al. [35]. In this respect, the samples were collected by using a sterile scalpel and wiped with a sterile cotton swab. Microscopic samples were collected from all Artefacts. Isolation of microorganisms was conducted through standard microbial culture procedures. The swab was placed in test tubes with 10 mL of mineral medium at pH 6.7. The tubes were transferred to the lab where 1 mL of each sample was diluted in 10 mL sterile water and shaken for 15 min. Resulting suspensions (about 0.5 mL of each sample) were inoculated in Petri plates by 5 cm in diameter, containing Malt agar medium [36] or Luria Bertani agar (LB) [37], and incubated for 7 days at 28 °C.

DNA-based molecular analysis

DNA extraction and amplification from isolated cultures

Genomic DNA from isolated cultures was extracted following the procedure described by Doyle [38]. For the identification of bacterial 16S rRNA sequences, DNA was amplified with the primer pair 341f/907r [39]. For the analysis of fungal sequences, fragments of about 700 bp in size corresponding to the ITS1, the ITS2 region, the intervening 5.8S rRNA gene and small portions of 18S and 28S were amplified with the primer pair ITS1 and ITS4 [40].

PCR amplifications were carried out on an estimate of 10 ng of extracted DNA, in a final volume of 50 μL containing 5 μL of 10X PCR buffer, 100 mM of deoxynucleotide

triphosphate, 2.5 mM of magnesium chloride, 0.5 mM of primers and 1U of Taq polymerase (Quiagen, Hilden, Germany). The PCR program consisted of an initial denaturation at 95 °C for 4 min and 30 cycles including 1 min of denaturation at 94 °C, 45 s of annealing at 56 °C and 2 min extension at 72 °C. A final extension of 7 min at 72 °C was followed by cooling at 4 °C. PCR products were inspected on 1% (w/v) agarose gels.

Denaturing gradient gel electrophoresis

Small fragments of the artefacts were collected with a sterile scalpel. Genomic DNA was extracted from these fragments using the procedure indicated above [38]. For denaturing gradient gel electrophoresis (DGGE) analysis, 200 bp fragments of the 16S rDNA were amplified with the same primer pair as above (341f/907r) and re-amplified using the same eubacterial specific primer 341f-GC, modified with a 40-bp GC clamp added to its 5′ end, as a forward primer [41] and, as a reverse primer, the universal consensus primer 518r [42] (which is upstream the 907r primer employed in the first PCR); for the analysis of fungal sequences, the same approximately 700 bp fragments were amplified using the same primer pair employed for isolated cultures (ITS1 and ITS4) and re-amplified with the same primer pair, except that the forward primer ITS1f-GC was modified with a 40-bp GC clamp added to its 5′ end [43]. All reactions were carried out as described in Michaelsen et al. [44]. For DGGE, we used an equipment DCode ™Universal Mutation Detection System Model 475 (BioRad, Berkeley, California). Electrophoresis was performed on 0.75 mm thick 6% polyacrylamide gels (37:1) with denaturing gradient ranging from 30 to 55% and 20–50% (100% denaturant contains 7 M urea and 40% formamide) for 16S and ITS rRNA, respectively, and submerged in 1X TAE buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA, pH 7). PCR products were applied to individual lanes in the gel. Electrophoresis conditions were 16 h at 100 V and 60 °C. Gels were stained for 30 min in 1X TAE buffer with SYBR Gold nucleic acid stain and visualized using a Fluor-S MultiImager and MultiAnalyst imaging software (BioRad, Berkeley, California). DGGE fragments were sequenced after excision from gel and re-amplification. Briefly, bands were excised, re-suspended in 20 μL of DNase free water and stored at 4 °C over night.

Sequencing of isolated microorganisms and DGGE fragments

The PCR products from the isolated cultures were purified with a QIAquick PCR purification kit (Qiagen GmbH, Hilden, Germany). PCR purified products and the majority of DGGE bands were sequenced with a 3130 genetic analyzer (Applied Biosystems, Foster City, California) and their sequences were edited and aligned using the Bio Edit software (version 7) [45]. Sequences were compared with those in the GenBank/EMBL/DDBJ sequence database using BLASTN algorithm available at the National Center for Biotechnology (NCBI, https://www.ncbi.nlm.nih.gov/refseq/). For community fingerprint comparison of different samples, we quantified allele richness (number of detectable bands) for both 16S and ITS rRNA in each lane and used automatic detection of bands by UVIdoc HD5 gel documentation system (UVITEC, Cambridge, UK). Sequences were attributed to species only if percentage similarities were > 96%.

Results and discussion

Environmental factors

Micro-environmental parameter values recorded in the rooms hosting the investigated artefacts (Table 1) may have contributed to favour microbial and insect growth on all the artefacts.

Relative humidity values reported for the rooms hosting the investigated artefacts (Table 1) are in all cases higher than recommended by official standard-defining documents [46]. Thus, excess humidity may have contributed to microbial and insect growth on all the artefacts, especially on Artefacts 3 and 4, which were exposed to even higher relative humidity values (Table 1). The same is true for temperature values (Table 1), higher than the recommended ones [46] for rooms hosting the Artefacts 3 and 4, which may have promoted microbial and insect growth on these two artefacts. The light intensity values measured in all rooms hosting the artefacts (Table 1) are the only values that fall within the ranges indicated as optimal [46].

Woods

The following histological characters allowed identification of the wood in each artefact:

Wood of Artefact 1: diffuse to semi-ring porous; radially orientated pore clusters; rays two- to four-seriate; height of rays very variable; ray cells small, axial oval; parenchyma apotracheal; perforation plates simple; spiral thickenings distinct; ray-vessel pits small and numerous (Fig. 2a–c). Based on these characters, the wood was attributed to a species of lime (*Tilia* sp.).

Wood of Artefacts 2 and 4: diffuse to semi-ring porous; pores solitary or in short radial files; rays uniseriate, average height 10 to 15 cells; ray-vessel pits large and simple; intervessel pits large; perforation plates simple (Fig. 2d, e). Based on these characters, the woods were attributed to a species of poplar (*Populus* sp.).

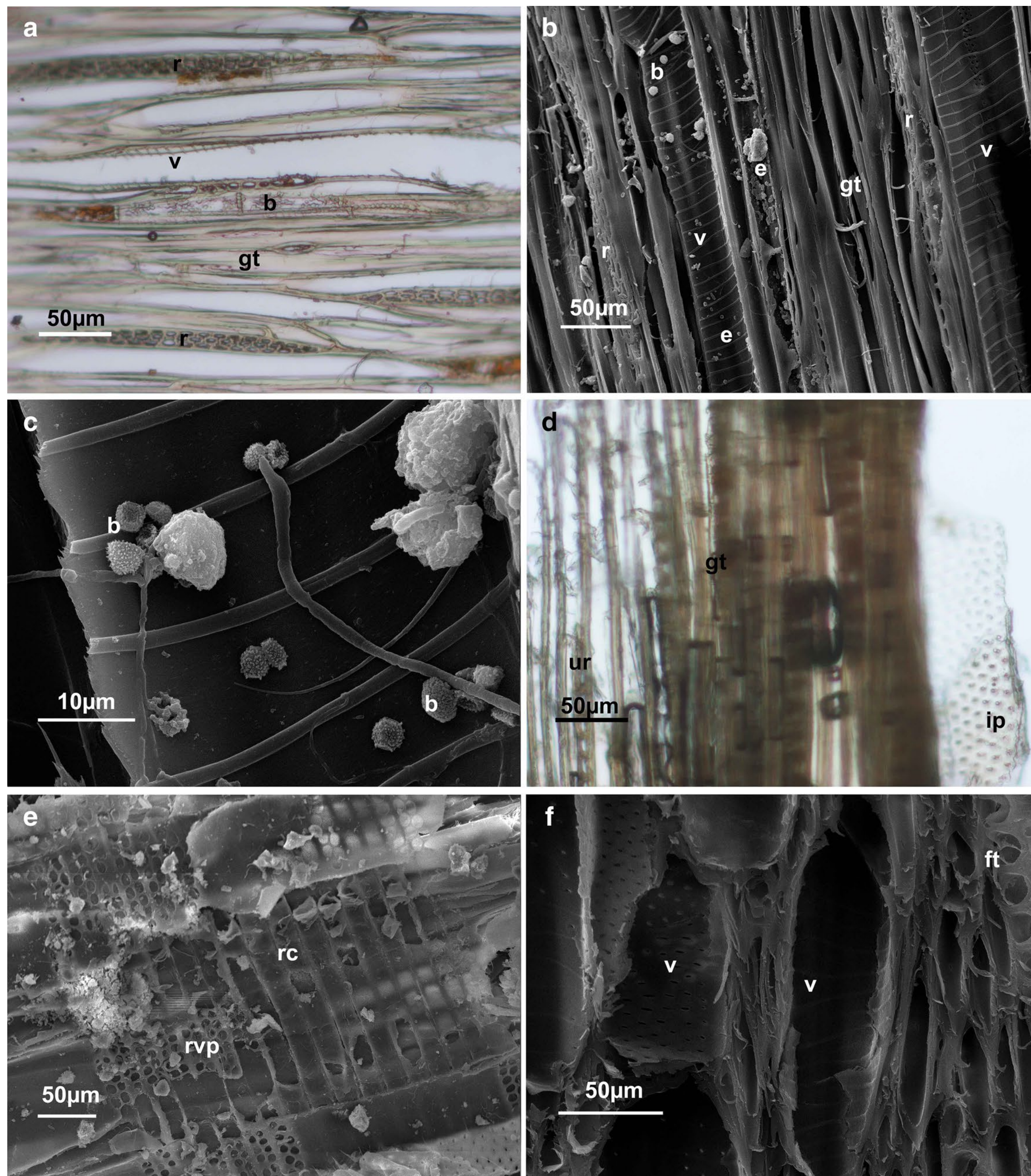

Fig. 2 **a–c** *Tilia* wood of the Artefact 1, **a** tangential section showing rays (r) two- to four-seriate, a vessel (v), thin walled ground tissue (gt) and biodeteriogens (b). **b** Wood surface showing two vessels (v), two rays (r) and ground tissue (gt); biodeteriogens (b) and eroded area (e) are present on vessel surface. **c** Detail of **b** showing biodeteriogens (b) on inner surface of a vessel. **d** *Populus* wood of the Artefact 2 Tangential section showing a vessel with intervessel pits (ip), ground tissue (gt) and uniseriate rays (ur). **e** *Populus* wood of the Artefact 4. Radial wood surface showing ray cells (rc) and large ray-vessel pits (rvp). **f** *Pyrus* wood of the Artefact 3 Wood surface showing vessels (v) and fibre tracheids (ft) (**a, d** light microscopy; **b, c, e, f** scanning electron microscopy)

Wood of Artefact 3: rays two-, three- or seldom four-seriate; ray cells round to slightly oval-elongated; fibre-tracheids present; perforation plates simple; fine spiral thickening occurs in the vessels (Fig. 2f). Based on these characters, the wood was attributed to a species of pear (*Pyrus* sp.).

The lime wood identified here in Artefact 1 by light and electron microscopy confirmed a previous report by light microscopy only [28]. Lime and poplar woods were identified in a number of other wood sculptures in southern Italy [32, 47] and pear wood is reported to be commonly used for preparation of various artefacts of reduced sizes, included

crosses [32]. Wood surfaces from all artefacts showed biofilms in the form of black patinas. Microbial cells were seen in some wood sections (Fig. 2a–c).

Insects

Dead insects were found on the Artefact 1 and on eight out of ten of the examined frames (Artefact 4). The other artefacts resulted free from insects. Restoration work carried out before the present study probably caused insect death on the Artefacts 1 and 4.

Data on the identification of insects occurring on the Artefact 1 were already published by two of the authors of the present work [28]. Insects found on this artefact were referred to the beetle species *Oligomerus ptilinoides* (family Anobiidae) [28].

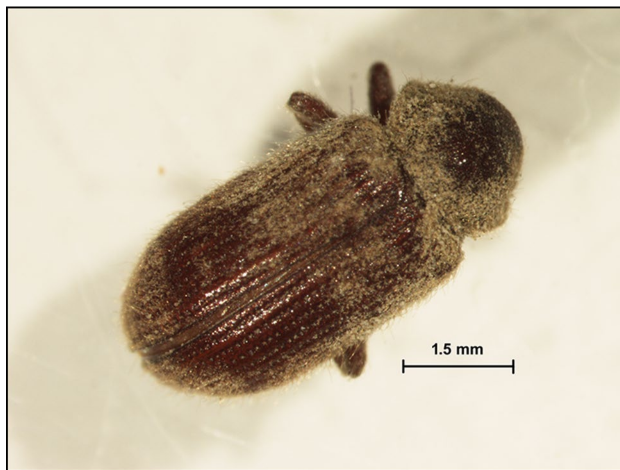

Fig. 3 The beetle infesting the Artefact 4 and identified as *Nicobium castaneum*

Insects found on the Artefact 4, examined in the present study, had body length of 5.8–6.2 mm, with brown setae, head recessed into the pronotum, robust mandibles; exit holes measured 1.5–2.5 mm in diameter and powdered frass had a gritty form. Based on such morphological features, the infesting insects were referred to the beetle species *Nicobium castaneum* (family Anobiidae) (Fig. 3).

Both *O. ptilinoides* and *N. castaneum* were reported as xylophagous pests on wooden sculptures in several churches in southern Italy and are known to prefer closed and humid environments [24, 26, 48]. Moreover, the presence of insect galleries, causing open spaces within the wood, was reported to promote fungal and bacterial proliferation, as insects digest cellulose thanks to the yeasts present in their midgut cecal epithelium [24, 26, 48].

Microorganisms

The microorganisms detected after culture-isolation, DNA extraction and amplification and those identified by DGGE of fragments of each artefact resulted the same, showing complete identity between the results of the two techniques.

The presences and absences of single microorganism species are reported in Table 2, separately for bacteria and fungi.

The profiles revealed a total of 11 species which were assigned to four species of bacteria and seven species of fungi (Table 2). Figure 4 shows the profiles (A: Bacteria; B: Fungi) with the highest diversity in microorganism species among all the sampling points.

Table 2 Band occurrences for each species of microorganisms identified in the four examined artefacts

Species	Similarity and GenBank Codes	Artefact 1 (Lime)	Artefact 2 (Poplar)	Artefact 3 (Pear)	Artefact 4 (Poplar)
Bacteria					
Bacillus cereus	98% KX035061	–	B4	–	–
B. mycoides	99% KX035066	–	B2	–	B2
B. subtilis	100% CP017314	B3	–	B3	B3
Microbacterium oleivorans	97% KU891044	–	B1	–	–
Fungi					
Alternaria alternata	99% AF404657	F1	F1	–	–
Aspergillus fumigatus	99% FJ878717	–	F2	F2	F2
A. versicolor	98% FJ878627	–	F4	–	F4
Cladosporium cladosporioides	99% FJ797611	–	F5	–	F5
C. oxysporum	99% JQ775499	–	F6	–	F6
Fusarium oxysporum	100% KU129006	F3	–	–	F3
Penicillium chrysogenum	98% DQ249212	–	F7	F7	F7

Occurrences (indicated by band names as in Fig. 4) are separately reported for bacteria and fungi. The type of wood of each artefact is indicated in brackets

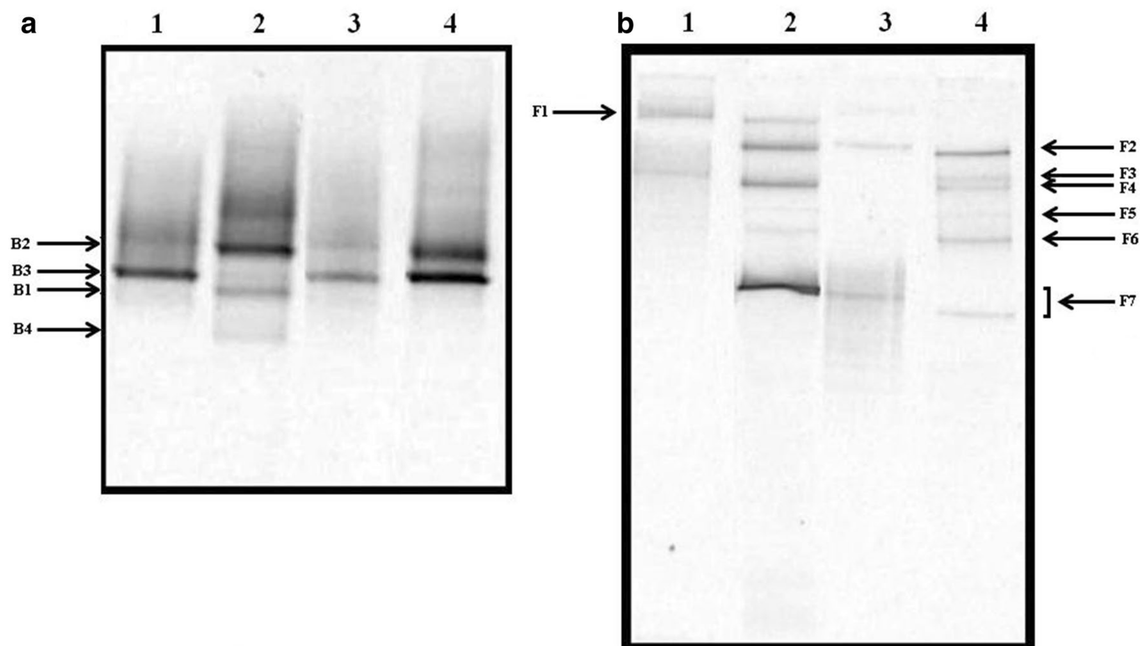

Fig. 4 One of the DGGE profiles, chosen as it shows the greatest diversity of bacteria and fungi as compared to others. Bacteria (**a**) and fungi (**b**) are in separate gels. Lanes: 1, artefact 1; 2, artefact 2; 3, artefact 3; 4, artefact 4. The numbered bands were excised and the results of species identification by sequencing the bands are shown in Table 2. B1–B4 shows bacterial DGGE bands and F1–F7 show fungal DGGE bands

Among bacteria, *Bacillus subtilis* was by far the most represented species, followed by *B. mycoides, B. cereus* and *Microbacterium oleivorans* (Table 2). Among fungi, *Penicillium chrysogenum, Alternaria alternata, Aspergillus fumigatus* and *Fusarium oxysporum* resulted the most frequently found species, whereas all other species were much less represented (*Aspergillus versicolor, Cladosporium cladosporioides* and *C. oxysporum*) (Table 2).

Table 2 also shows that a higher number of species of colonizing microorganisms occurred on Artefacts 2 and 4, whereas Artefacts 1 and 3 were less colonized. As far as the frequency of species on the artefacts is concerned, among the bacteria, *Bacillus subtilis* was the most frequent species, occurring on three artefacts, whereas *B. mycoides* occurred on two artefacts and both *B. cereus* and *Microbacterium oleivorans* on one artefact only; among fungi, *Penicillium chrysogenum* and *Aspergillus fumigatus* were the most frequent species, with each of them being present on three artefacts; each of the other species was present on two artefacts only. Table 2 also shows the occurrence of species by type of wood. In this respect, *Bacillus subtilis* colonized three types of wood, whereas each of all other microorganisms colonized two or one type of wood. Finally, Table 2 shows the species that occur together on the substrates more frequently than others. In this respect, the most frequent association of species is represented by *Aspergillus fumigatus* and *Penicillium chrysogenum*, occurring together on three artefacts.

We did not compute abundance of microorganisms as function of band intensity (as, for example, indicated in Formin et al. [49]) because we were primarily interested in identification, rather than quantification, of microorganismal taxa. In addition, four artefacts, even if with six sampling points each, are too small a sample to be reliably treated in a statistical fashion.

All identified microorganisms, with the exception of *Microbacterium oleivorans*, are known to be biodeteriogens of art-works [50].

Among bacteria, three out of four identified species belonged to genus *Bacillus* (Table 2). *Bacillus* is a genus including hundreds of strains [7], most of which were detectable in bacterial communities on indoor wood artefacts where they revealed biodegradation properties [21], including cellulolytic activity [22]. The prevalence of *Bacillus* on wood could be also due to the presence in its genome of genes encoding cellulose degrading enzymes, as reported for at least some species of this genus [51, 52].

It is also known that the bacteria responsible for erosion degraded the secondary wall layers using the cellulose and hemicellulose of the wood [21, 53, 54]. As a result, the residual material of the cell wall usually has a porous appearance [32]. This type of alteration was in general observed on the artefacts investigated here, in particular on Artefacts 1 and 4, which were colonized by *Bacillus* and presented a greater degradation state (Fig. 2e).

Among the identified *Bacillus* species, as well as among all identified species, the most abundant one was *B. subtilis* in terms of occurrence on the various artefacts and types of wood (Table 2). This predominance can be explained by the presence of *B. subtilis* in various environments and by its ability to survive in harsh conditions by forming resistant endospores [55, 56]. *Microbacterium oleivorans* identified on the Artefact 2 only (Table 2) is a bacterial species described from oil-containing environments [50]. Its occurrence on artefacts has not been reported so far. It is noteworthy that other species of this genus are known for colonizing art-works [11] though, and that *Microbacterium oleivorans* can degrade crude oil [50], that is an organic compound present in dyes used to paint the investigated artefacts and that may have contaminated the wooden parts examined in the present work.

Among the identified fungi, the genera *Alternaria, Aspergillus, Cladosporium* and *Penicillium* (Table 2) were reported as always predominant in indoor and outdoor environments [28, 57] and as commonly attacking wooden art objects [15, 18, 22, 58–63]. Three of the four genera, *Aspergillus, Cladosporium* and *Penicillium*, are filamentous fungi likely to be the main agents in the alteration of the colour and in the structural deterioration of wood due to their production of cellulolytic and xylanolytic enzymes that degrade the wood fibres [18, 22, 64]. Fungi like *Aspergillus* and *Penicillium* may act in low humidity conditions, such as those of our artefacts; they grow in the S2 layer of the secondary cell wall, by enzymatically dissolving cellulose and hemycellulose; they also display a strong amylasic, pectinasic and xylanasic activity and interact with fungi by stimulating their decomposition activity. Thus, the species of these genera may be involved in the formation of the black patinas observed on wood surfaces of all artefacts investigated here [64–67]. In addition, *Aspergillus, Fusarium* and *Penicillium* species (Table 2) may have contributed in causing the black patinas, since they are reported to be superficial chromogenic fungi able to grow in the first layers of cells and to a depth not greater than 1 mm through natural openings or traumatic injuries [64]. Many insects attack wood only after the wood has been altered by fungi [67].

Most fungal species are known to be polyphagous and ubiquitous. Besides present reports on wood decay in artefacts from Naples, in Italy, a series of other reports are indeed available for different geographical areas [1], included reports of species of the genera *Cladosporium* and *Penicillium* detected on historical wood in Morocco [68] and of the genera *Alternaria, Aspergillus* and *Penicillium* isolated from wooden objects in Iran [69].

As far as the relationships here reported between bacteria and fungi are concerned, it is notable that bacteria grow on the surface of the wood degraded by fungi; these increase the amount of nutrients produced by the decomposition of organic compounds [70]. Then, some bacteria not only have an influence on the permeability of the wood [23], but also attack the structure by working in association with other bacteria or fungi, preparing the wood to fungal attack [14]. It was also proposed that fungi are first colonizers of paintings and wood and that their metabolic products are used by bacteria [7]. In addition, according to Ciferri [2], the bacteria of genus *Bacillus* cannot grow on paintings by themselves, but only if fungi as *Aspergillus* and *Penicillium* promote their survival. Other studies [52, 64] have shown that certain bacterial species induce the degradation of wood according to the ratio that establish with fungi and other xylophagous bacterial species.

Relationships between bacteria and insects were also investigated in the past [13, 20, 23, 71, 72]. Bacteria are reported to cause the formation of thin galleries within the secondary cell wall of wood [52, 64] and this mechanism is facilitated by the presence of xylophagous insects belonging to Anobiidae [23, 26, 48], the family that includes *Oligomerus ptilinoides* and *Nicobium castaneum*, the species found on the Artefacts 1 and 4.

The higher diversity of microorganisms discovered on Artefacts 2 and 4, both made of poplar wood, as compared to the lower diversity on the other two artefacts, made up of lime and pear woods, is tentatively interpretable in the light of the different types of wood. Poplar wood might have favoured a more diversified microbial growth due to its greater softness (density 0.3–0.5 g cm^{-3}), as compared to more compact and hard lime (density 0.65 g cm^{-3}) and pear (0.6–0.7 g cm^{-3}) woods [34].

The fairly high rates of relative humidity and temperature in the rooms where Artefacts 3 and 4 are exhibited (Table 1) may have also been involved in causing the higher biodiversity found on the Artefact 4 (Table 2), where they may have stimulated, in conjunction with the presence of the softer poplar wood, the diversified microbial colonization.

All the results obtained in the present work, besides giving a contribution to the knowledge of micro-habitats represented by biofilms, appear also of interest in the field of conservation of wooden art works colonized by biodeteriogenic organisms. In this respect, they represent a starting point for finding strategies to adopt in the field of cultural heritage restoration. The knowledge of biodeteriogenic species and the type of colonized substrates is often crucial for the choice of suitable techniques to apply in the removal of biofilms.

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