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MINI-REVIEW

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Physiology of biodeterioration on canvas paintings

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The study of the physiological action of microorganisms in artistic materials is one of the most interesting topics in biodeterioration nowadays. Pathologies and illnesses of organic and inorganic materials provoked by microorganisms can be treated by experts by a variety of preventive interventions. *Artistic medicine* encompasses the monitoring of the exhibition and storage of art, as well as proper environmental conditions and the regular cleaning of museums. Biodeterioration control is essential in order to prevent fungal and bacterial contamination in artwork. Biodeterioration of canvas paintings is a complex phenomenon, not well-known at the moment. Canvas paintings are created by several artistic techniques on textile supports that are not always kept in the best conditions, and the best parameters of preventive conservation are often not applied. Therefore, we need to research the agents and the main causes that provoke canvas painting biodeterioration. By applying new methodologies, we can identify the alterations and the treatments needed in order to manage the diverse materials employed in artwork correctly. Herein, we review the causes of biodeterioration that affect artwork, especially art created on textile supports. We also study the alterations of the natural filmogenic materials employed in traditional pictorial techniques, such as agglutinants and protection layers, and the biodeterioration agents that impact them. Additionally, we review current scientific methods employed for the identification of microbial species, and the types of alterations of the materials where the organisms grow. Finally, we summarize the different biocides and preventive conservation treatments that are currently employed.

KEYWORDS
biodeterioration, canvas painting, conservation, microbiology, molecular biology

1 | INTRODUCTION

Our understanding of the processes of biodeterioration in artwork has experienced a huge evolution in recent years, due to the development of new approaches in the field of conservation and restoration of art. Essential methods have been developed by research groups from different scientific and cultural environments (Caneva, Nugari, & Salvadori, 2005; Piñar, Tafer, Sterflinger, & Pinzari, 2015; Sterflinger, 2010; Sterflinger, Ettenauer, & Piñar, 2014). Therefore, the biological aspects of art restoration currently are an important issue, involving the study of environmental conditions, and the microbiology and vegetal biology of artwork, and cultural heritage items. Much research is currently focused on the action of vegetal organisms on the inorganic materials of facades, fountains, stone sculptures, glass, archaeological materials, and cave paintings (Caneva, Nugari, Pinna, & Salvadori, 1996; Carmona et al., 2006; Ciferri, 1999; De Los Ríos, Cámara, Wierzchos, & Ascaso, 2008; Jurado, Sánchez, & Saiz, 2008; Koestler, Koestler, Charola, & Nieto, 2003; Saiz & Gonzalez, 2007; Sterflinger, 2010; Stomeo, Portillo, González, Láiz, & Saiz, 2008; Zammit, Psaila, & Albertano, 2008). In the specific case of mural paintings there are a number of studies that analyze microbiological deterioration, involving mostly inorganic materials. However, there are only few studies on biodeterioration in easel and canvas paintings. This review addresses the causes, processes, and treatment of biodeterioration in easel and canvas paintings.

Pictorial work achieved on canvas paintings has a complex constitution, with a mix of inorganic and organic materials, arranged in thin layers of diverse character and functions. Description of alterations has been provided mostly on a macroscopic level but not at the microscopic level. However, microscopic studies are necessary in order to understand biodeterioration processes in organic materials. The textile support of canvas paintings provides the main organic material for fungal and bacterial growth, and can support microbiological alteration processes in a variety of environmental conditions (Caneva, Nugari, & Salvadori, 2000). Accordingly, the enzymatic action and the production of substances generated by fungus and bacteria on canvas painting have been studied by research groups in the fields of organic chemistry, pharmacy, food microbiology, and environmental sciences. The importance of biotechnological approaches in this field cannot be underemphasized. Biotechnology research employs powerful analytical techniques, and the use of molecular biology tools also has become indispensable to identify microbial species that participate in the alteration processes (Sterflinger, 2010). In this regard, there has been much progress in the description and diagnosis of the deterioration provoked by microorganisms, mainly by fungi and bacteria.

Regarding chemical-analytical and microscopic techniques, the use of Field Emission Scanning Electron Microscope (FESEM) allows researchers to identify processes occurring in the interior of the pictorial stratums. New graphic models have been developed with FESEM studies (Poyatos, 2007) that have supported settled theories of literature (Figure 1).

Experimental innovation is essential for advancing research in the field. One approach is to recapitulate artistic material biodeterioration processes in the laboratory. These studies are performed in the test tube, where microorganisms are inoculated into different materials and pictorial supports, then subjected to the appropriate environmental conditions. Subsequent assays examine the progress and alterations (Inoue & Koyano, 1991; López et al., 2013; Mandana & Nuruladidabt, 2014; Paner, 2012; Pavić et al., 2015; Romero, Martín, López, Ramos, & Bolivar 2010; Seves, Sora, & Ciferri, 1996). These studies allow us to analyze which processes take place as a consequence of microbial action on a canvas painting, and also to compare them with alterations that occur because of the non-biological degradation of the constituents of the materials. Hence, we could find the specific treatments against the different microorganisms that grow in the art materials.

However, we want to emphasize in this review that while much research has focused on the study and analysis of the diagnosis of biodeterioration, little has been accomplished in producing new approaches to conservation. This reflects the difficulty of the methodologies in applying effective treatments on canvas paintings without damaging the artwork. In this regard, we review the environmental conditions that need to be taken into consideration in a preventive conservation. We also examine the biodeterioration of canvas paintings, specifically in the textile support and filmogenic materials, in order to find new approaches for controlling microbial growth. Finally, we summarize the latest methodologies and treatments that provide successful therapies for biodeterioration in artwork.

1.1 | Environmental conditions

An understanding of biodeterioration and its causes requires the study of the relationships between microorganisms and their environments. A study of biodeterioration must relate the different biological populations to the physical-chemical conditions of the artwork and its surroundings. Herein, we should imagine a canvas painting as a patient, affected by a specific infection related to a specific microclimate. In biodeterioration we also have diverse "illnesses" associated with different regions or climates. For example, in tropical regions it is easier for a museum to receive an infectious plague. There is thus an urgent need to achieve new parameters of monitoring and control of environmental conditions as preventive measures toward biodeterioration processes (Molina & Borrego, 2015; Sterflinger & Pinzari, 2012).

Ciferri (1999) concluded that if environmental parameters are not controlled, the microbial communities of two paintings made of the same materials, but displayed in different places, will vary significantly, especially in the case of areas with tropical climate. The high levels of some parameters in warmer climates can significantly shorten painting lifetimes. These parameters exacerbate the damage provoked by atmospheric contamination, biological attack and natural aging. Caneva et al. (2000) in the Laws of Liebig and Shelford, explained the elements that inhibit the presence of a specific biological species. They also studied the factors on which the growth and dynamics of the biological populations and microbial communities depend, including soil, materials of the substrate, and environmental factors such as water, chemical composition of the air, humidity, temperature, light, and contamination. Some of the compounds that could be used as an energy source for microorganisms are the air pollutants sulfur dioxide and nitrogen oxides (Nuhoglu et al., 2006).

Biodeterioration has been evaluated in relation to the ecological space/time in which the artwork is situated (Dornieden, Gorbushina, & Krumbein, 2000). The elements that inhibit the onset of a biological attack and the conditions needed for the development of each biological species on a piece of art are reviewed (Villarquide, 2005). Normally, one of these inhibitory factors is sufficient to control the appearance of biodeterioration. The specific elements important for development of microorganisms and other biodeterioration agents are detailed in Figure 2.

All the biological agents that can affect an artwork will damage it depending on environmental conditions. Therefore, it is essential to monitor these elements (Figure 2) in order to avoid damage such as increased fragility, disconnection, change of color, or complete destruction of the canvas painting.

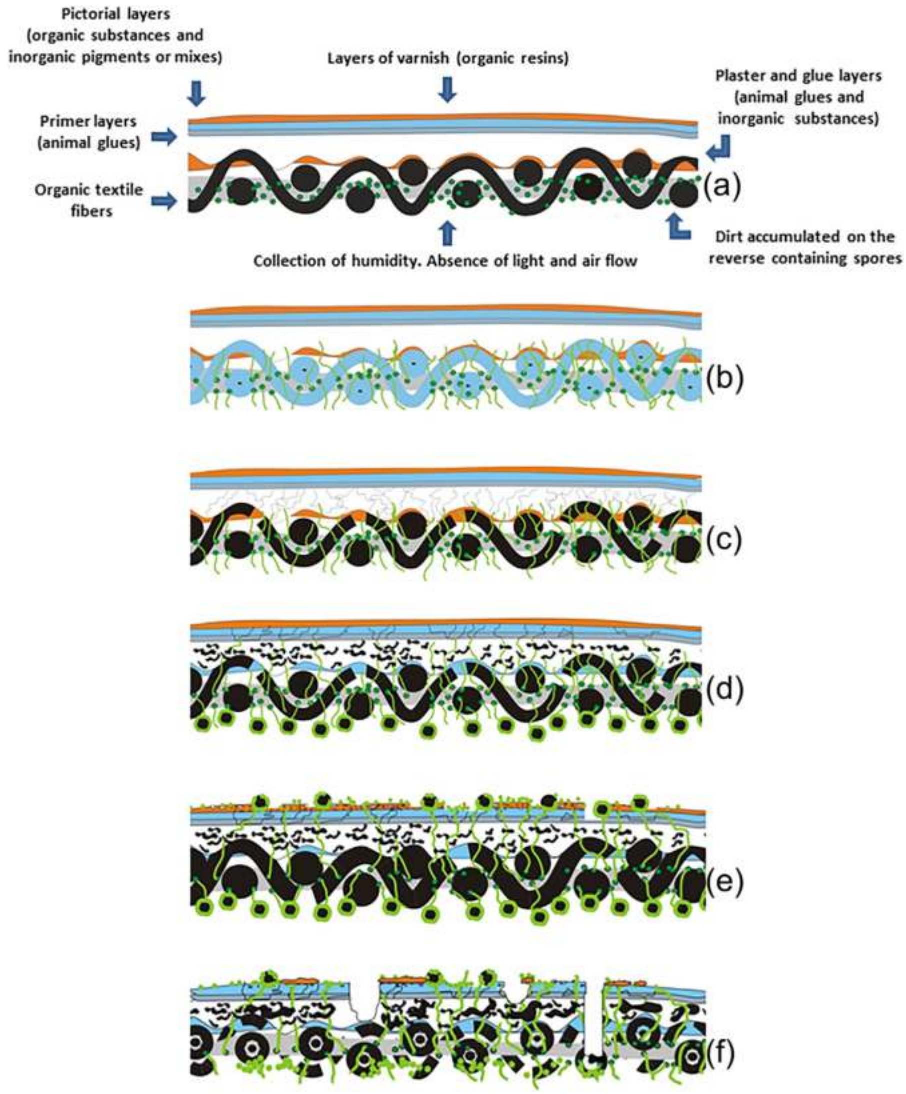

FIGURE 1 Graphical model of biodeterioration processes on a canvas painting. (a) Stratigraphy of a canvas painting. (b) Hydration of the fibers. (c) Degradation of the layers of plaster and glue, and general weakening. (d) The glues are hydrated, weakening the plaster and glue layers, and causing the pictorial layers to crack. The hyphae rise to the surface through the reverse. (e) Alteration of the varnish layers and adjustments in the other layers. Superficial dispersion of the spores. (f) The final stage involves destruction of complete layers and disintegration of the textile support (Poyatos, 2007)

1.2 | Canvas painting biodeterioration

There are several metabolic processes that happen in artistic materials. Microorganisms employ autonomous activities to metabolize artwork, and biodeterioration processes can happen in different ways depending upon the type of metabolic activity, either heterotrophic or autotrophic. First, an essential distinction must be made between organic and inorganic materials. There are many organic materials in canvas paintings that are subject to biodeterioration.

Analyses can monitor the activity of microbiological agents and alterations of artistic support on canvas painting, and on the filmogenic components, thus delimiting each type of organic material and the microorganisms in this support (Agrawal, Dhawan, & Garg, 1989; Bolívar, 1995; Bravery, 1988; Evans, Wales, Bratt, & Sagar, 1992; Gargani, 1968; Gaylarde & Moreno, 2006; Giacobini and Firpi, 1981; Ross, 1963; Strzelczyk, 1981). Other studies deal directly with the nature and composition of the organic materials of canvas paintings, and either focus on the composition of the textile support, or the agglutinants, pigments and protection layers of the pictorial substrate. Thus, the phenomenology of the alterations can be extremely diverse between the obverse and reverse of a canvas painting. These approaches are best appreciated in several phases. These studies provide essential data about the analytic methods employed, and as they do not employ destructive analyses, they therefore are an important reference in research (Giacobini, Nugari, Pietrini, & Ricci, 1983; Mc Carthy, 1987).

Vázquez and Alonso (1990) studied the action of fungi on Spanish easel paintings of the 18th century. They were able to distinguish between mechanical and chemical effects, and suggested fungicidal and fungistatic treatments. The action of bacteria and

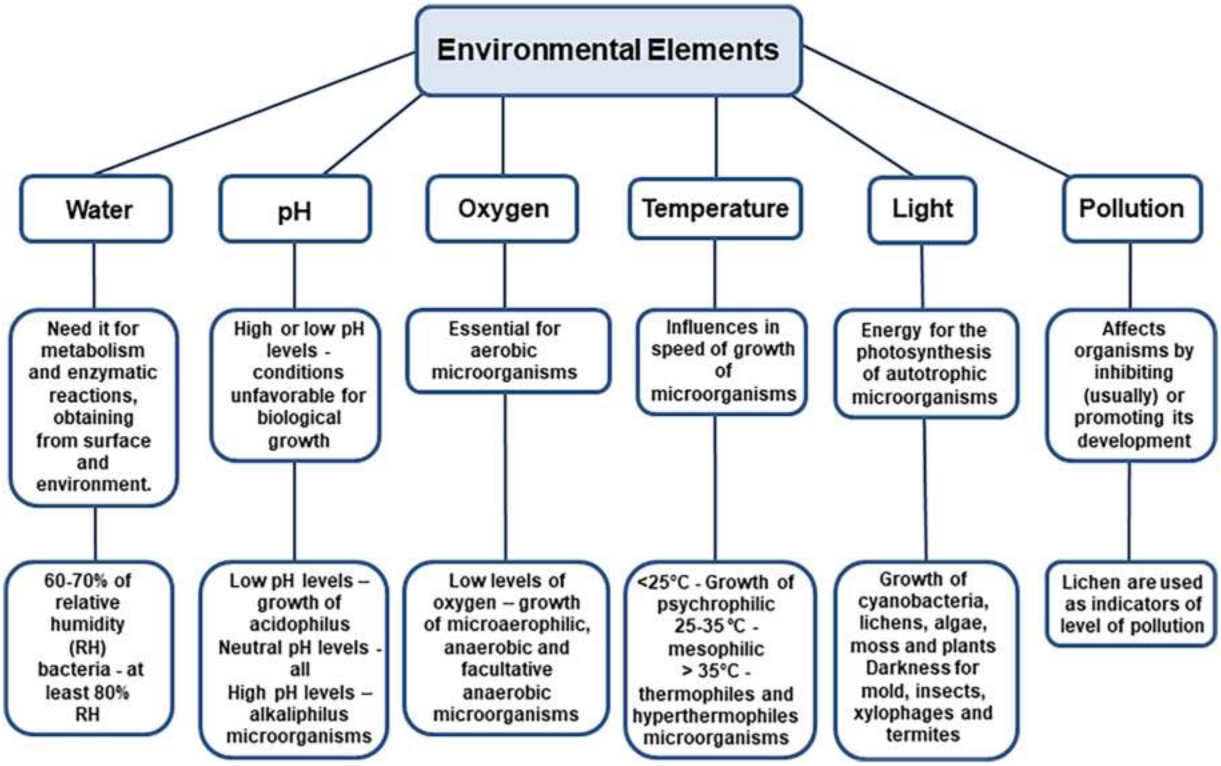

FIGURE 2 Environmental elements important for biodeterioration

fungi is recognized by the appearance of color spots and texture changes that significantly modify the pictorial layers (Venegas & Pardo, 1995). An experimental laboratory study carried out by Seves et al. (1996) and Seves, Romano, Scilione, Maifreni, and Sora (2000) with pictorial supports of traditional materials, such as linen canvas prepared with layers rich in animal glues and linseed oils, then inoculated with different fungi and bacteria, allowed identification of the species of bacteria: *Bacillus pumilus*, *Bacillus circulans*, *Bacillus cereus*, *Micrococcus luteus*, and the fungi *Aspergillus niger* and *Penicillium chrysogenum*. Other studies also value the features that the paintings have, achieving a detailed relation between the level of biodeterioration of each organic and inorganic material, and the treatments performed (Cortet, 1988; Guillaume-Chavannes, 1989). Nicolaus (1999) carried out a complete study of biodeterioration in paintings, and described the conditions of development, the beginning of colonization, and the fatal consequences of alteration of the physical and chemical structure of the pictorial work. His study involved generating images of the damage created on the surface of paintings on a macroscopic level.

Microbiological alterations in easel paintings can be caused by the bacterial genuses *Pseudomonas*, *Alcaligenes*, *Bacillus*, *and Flavobacterium*, as described by Ciferri (1999). The bacteria can enable posterior colonization by common fungi, with *Aureobasidium (Pullularia) pullulans* as the main biological agent. The pigmentation of mycelia also has been studied, such as in the case of *Dematiaceae*, that causes more aesthetic superficial damage than other species (Caneva et al., 2000). The same group also analyzed the organic substances of paintings that could be a nutrition source for microorganisms.

Concerning processes occurring in filmogenic material, an experimental study examined the biological alterations that appear in the protection layers made by terpenic resins, specifically by dammar resin (Romero et al., 2010). This study was carried out with a series of resin-containing test tubes, into which diferent fungi and bacteria were inoculated. The researchers then followed the deterioration of each resin by the action of microorganisms. Pavić et al. (2015) achieved an interesting experimental study about the potential of degradation of diverse microorganisms in several artistic materials, specially in pigments. They analyzed the solubility capacity that each species could have in zinc white, ivory black, ochre yellow, cobalt green, and cadmium red. All of the studies employed Scanning Electron Microscopy (SEM) in order to visualize the microbial structures, alterations, and excreted substances.

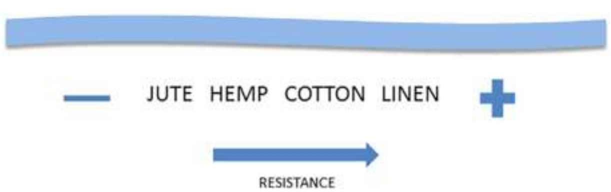

FIGURE 3 Resistance scale for biological alteration of textile fibers (Caneva et al., 2000)

The heterogeneity of alteration is one of the main characteristics of biodeterioration of canvas painting. The diversity of substances and materials employed in the artwork are determinants of the typology of degradation and the physiology of the microbiota. The type of microbiota will be constrained by the nutrients and environmental conditions. These studies and the ones reviewed below allowed the findings of new research protocols in the field.

1.3 | Biodeterioration of textile supports in pictorial works

The physical-chemical conditions of the textile support are a prime determinant of biodeterioration of canvas paintings. In European artistic work, linen and hemp were the main fibers employed, dependent on the specific geographic area, until cotton emerged with the industrial revolution. These materials respond in diverse ways to hygroscopic conditions and percent cellulose, inter alia. The type of “pathology” can differ by the relative contributions of these properties, that will determine at the same time the metabolic function and activity of the microorganisms and the type of alteration that appears.

In studies on the biological origin of deterioration on textile pictorial supports, Schlegelb and Jannasch (1981) described the degradation of cellulose fibers by bacteria such as Cytophaga and Sporocytophaga. Other species referred to are Trichoderma viride, Chaetomiun verrucaria, and Penicillium funiculosum (Montegut, Indictor, & Koestler, 1991). Nicolaus (1999) explained how fungi and bacteria degrade cellulose in artwork. The decomposition of cellulose directly influences the appearance of decay that is similar to seen in the oxidation of textile supports. The textile fiber loses its consistency and elasticity, becoming easily breakable and deformable. Nicolaus also highlighted the importance of monitoring climate conditions in buildings where the artwork is exposed, as a high relative humidity, or condensed water is needed for the growth of microorganisms.

Caneva et al. (2000) proposed that biological attack depends on the percent content of cellulose, lignin, and other organic components of the artwork. The higher the percentage cellulose the artwork contains, the more resistant it will be to attack. Materials can be classified according to the quantity of lignin. The materials that have higher lignin content are more resistant to biological attack, while the ones with lower quantities will be less resistant to attack. The ordered classification is: jute, hemp, cotton, and linen (Figure 3). There are some microorganisms that disturb the biodegradable features of the fiber of cellulose, such as polymerization grade, length of the chains, crystallinity, and orientation. Mechanical, chemical, or photochemical damage to fiber can increase the susceptibility to biodeterioration and modify the structural features. Some of the most important microorganisms that provoke such biodeterioration are: *Alternaria*, *Aspergillus*, *Fusarium*, *Memnoniella*, *Myrothecium*, *Neurospora*, *Penicillium*, *Scopulariopsis*, *Stachybotrys*, *Stemphylium, and Chaetomium* for fungi; and *Cellvibrio*, *Sporocytophaga*, *Myxococcoides*, *Cellfalcicula*, *Microspora* for cellulosic bacteria, and also the anaerobic bacteria *Clostridium* sp. There also are insects that contribute to biodeterioration in textile supports (Sánchez & Bolívar, 2003; Szostak, 2004). These insects promote the growth of microorganisms that cause fiber coloration.

A significant research finding was achieved by Villarquide (2004, 2005), who showed the dependence of alteration on cellulose, as this substance is the primary component of textile supports. The studies described the percentages of organic substances, including cellulose and lignin, that form textile supports and also are determinants of the extent of microbial action (Table 1). Villarquide's studies emphasized the importance of analysis of environmental conditions and physical-chemical changes on the support as they relate to the processes of biodeterioration. These reports also described the types of enzymatic degradation, mechanical damage, optical alterations, and the action of acids and bases on microbial metabolic processes.

TABLE 1 Percentage of organic substances in textile support of canvas painting (Villarquide, 2004)

Fibers	Cellulose (%)	Lignin (%)	Other subtances (%)	Humidity absorption (%/100% relative humidity)
Linen	76–88	0.2–0.5	Hemicellulose: 19	13
			Pectin: 6	
			Wax: 3	
Hemp	70–80	2–6	Hemicellulose: 16–17	12
			Cutin: 2–4	
			Pectin: 4–8	
			Wax: 0.5–1.5	
			Ash: 1.5–2.5	
Cotton	90	2	Hemicellulose: 4–7	40
			Wax and fat: 0.5	
			Minerals: 0.5	
Jute	64–78	14	Hemicellulose: 29	14
			Wax: 1	

Some filamentous fungi are agents of damage to canvas paintings because they can dissolve cellulose fibers through the action of cellulolytic enzimes. The fungi also can decolor supports, and degrade glue, tinctures and oil layers. Microorganisms also can hydrolyze collagen fibers and other proteinaceous materials, and can modify inorganic components, causing pigmentation and organic acid production (Sterflinger & Piñar, 2013). A textile support is in many respects an ideal substrate for initiating biodeterioration. We therefore need to consider artwork maintenance and preventive conservation, as well as the addition of other organic materials in restoration procedures carried out in response to traumatic environmental events, such as floods (Capodicasa, Fedi, Porcelli, & Zannoni, 2010).

1.4 | Biodeterioration in filmogenic materials

Filmogenic materials are the second contribution of organic material in the structure of a painting. The types of materials employed are very different and are in diverse amounts, depending of their technical function. Filmogenic materials are listed in Table 2.

The first material to degrade in the process of biodeterioration is the glue that impregnates the reverse of the support (Berovic, 2002). The glue is applied as a first layer of printing, and acts as a sealant. In this case, the influence of environmental conditions is decisive, as glue becomes a culture medium following hydrolysis and serves as a nutrient for microorganisms. Villarquide (2005) showed the consequence of biodegradation, where glues and oils suffer, especially when there are dust deposits and humid conditions, causing the loss of adhesive and agglutinant capacities of the materials. The loss of color on oil paintings provoked by certain bacteria was analyzed by Khijniak, Medvedeva, Golikov, and Dick (2005). This study revealed the chromatic effects with the degradation of oils, as well as that the intensity of the darkening processes depends upon the species of bacteria and incubation time.

Natural resins employed as filmogenic materials in the protection layers also are sensitive to biodeterioration. There is one study on resin acids, specifically of the colophony resin that is located in paper pulp (Liss, Bicho, & Saddler, 1997). Other research studied the alterations that could affect the varnish, in which funguses are the source of whitening and weakening of the protection layer on canvas paintings (Calvo, 2002). However, resins exhibit a high resistance to biological attack, such that if it occurs it will be localized on the overlap dirt. Resins can be classified as: i) high resistance resins, such as terpens and acrylics; and ii) medium resistance resins, such as vinyl and alkyd. The authors also mentioned that mold is visually similar to pomades, although they can be distinguished by cleaning in water, in which mold is easily eliminated (Villarquide, 2005; Weirich, 1998).

Concerning the biodeterioration of terpenic resins for elaboration of protection layers, we highlight the work of Romero, Poyatos, Martín, and Bolívar (2008); Romero et al. (2010) and Romero et al. (2014). They analyzed sandarac, Manila copal, dammar, and Venice turpentine, and assessed the changes in the chemical composition of the resin in test tubes as a consequence of treatment with different microorganisms. They performed several techniques of organic substance analysis, including gas chromatography. It is essential in carrying out studies on artistic materials to determine the degree of alteration, the nutrients used by the microorganisms in each material, the excretion substances, and the physical-chemical changes provoked by the growth of each species of microorganism.

1.5 | Analytical methods

The employment of different microscopy techniques has been indispensable in the study of biodeterioration. A complete analysis of the structure of artistic materials and microorganisms can be accomplished using complementary microscopic techniques. A number of studies have applied specific analytic methods, such as SEM, chromatography, or electrophoresis for the identification of the chemical composition of materials and the effects of biodeterioration on these materials.

In the 1970s, the first reports appeared that used SEM as an analytic method for the observation of microbiological alterations on canvas paintings (Bassi & Giacobini, 1973). The researchers were able to identify the origin of the agents, and the microorganisms. These studies also highlighted the importance of the nature of the artistic support in relation to the deterioration, and emphasized that the type of biological degradation in a canvas can be distinguished from other general physical-chemical degradations. Subsequent studies revealed the effect of microbiological action on pictorial layers, and highlighted the importance of identifying the microbes that cause the alterations. Techniques employed included optical microscopy, transmission electron microscopy (TEM) and SEM, as well as techniques for quantifying levels of microorganisms (Giacobini, Roccardi, Bassi, & Favali, 1986; Giacobini, Pedica, & Spinucci, 1989). These protocols provided the groundwork for general and specific studies in the future.

Several SEM-based analyses subsequently were published. Maifreni, Romanó, and Freddi (1991) studied the microbial pollution of textile pictorial supports using SEM as analytical method. Other authors carried out studies focused on biodeterioration provoked by *Fusarium* sp. (Gu, Ford, Berke, & Mitchell, 1998). SEM also has been effective in analyzing oxidation in paintings caused by fungi.

TABLE 2 Composition of the natural filmogenic materials employed on canvas paintings (Fuster, Castell, & Guerola, 2004)

Natural filmogenic materials	
Proteins	Glues and animal gelatins, vegetal proteins, casein, egg and albumin
Polysaccharides	Flours, rubber and vegetal mucilage, cellulose, amides
Lipids	Oils, wax
Terpens	Natural resins, lacquer rubber

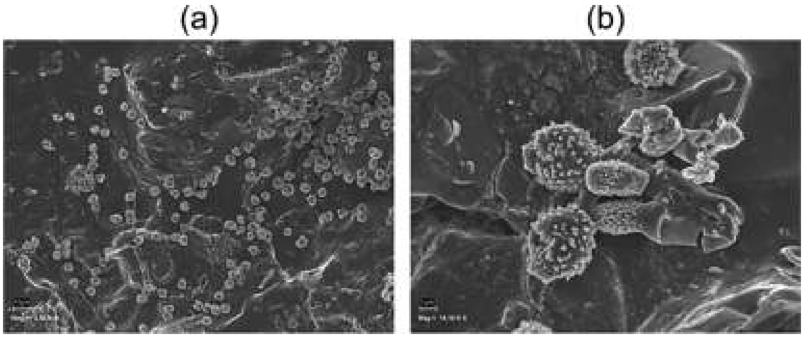

FIGURE 4 (a) Deposits of spores of *Aspergillus niger* in ground layers. (b) Hyphae of *A. niger* provoking alterations of the filmogenic material

Chromatography also has been used to identify organic and excretion substances in filmogenic materials (Arai, 2000). Pinzari, Pasquariello, and De Mico (2006) visualized the cellulosic fungi that affect textile supports on canvas painting. These studies collectively provided deeper insight on the the alterations of the internal structures in pictorial works.

FESEM was employed to analyze samples from the reverse and obverse sides of canvas paintings that were highly degraded. In these studies, the hyphae and spores of different species of fungi were observed inside the pictorial strata. As an example, Figure 4 shows deposits of spores (Figure 4a) and hyphae (Figure 4b) of *A. niger* in the ground layers of a canvas. Poyatos (2007) and Poyatos, Bolívar, Martín, Fernández, and Romero (2006) also visualized the consequences of the physical-chemical action provoked by the enzymatic action of microorganisms as a result of change in environmental conditions. With these results Poyatos proposed a graphic model (Figure 1) that explains the different phases of growth of the microorganisms, from the reverse to the obverse of a canvas painting.

De Los Ríos et al. (2008) analyzed the use of different microscopes for biodeterioration research. SEM/Back-Scattered Electrons (SEM/BSE) was employed for inorganic materials, providing images of the alterations. Low-Temperature Scanning Electron Microscopy (LTSEM) was used to localize humidity. Confocal Laser Scanning Microscopy (CSLM) allowed determination of the spatial distribution of microorganisms. Finally, TEM allowed investigation of microbial ultrastructure and also the structure of the inorganic materials with alterations, allowing the in situ study of microorganisms inside samples and in ecological niches. These microscopic methods are now used in combination with molecular biology techniques.

The dissecting light microscope is the best tool employed for studying superficial growth of microorganisms. The results of microscopic analysis are critical in making decisions about methods and cleaning treatments (Sterflinger & Pinzari, 2012). Pavić et al. (2015) described the impact of bacterial diversity on contemporary canvas painting, and employing SEM to observe samples of the reverse of the paintings, and determining the presence of subaerial microorganisms.

1.6 | Molecular biology techniques

The application of molecular biology to the study of biodeterioration is rapidly developing. Molecular biology tools are now indispensable for the accurate identification of microbial activities in artistic materials. Herein we will review the most important studies achieved to date in the field.

The development and employment of techniques of identification of microorganisms by DNA sequencing were originally described by Ciferri (1999), who argued that these techniques would allow a more accurate and complete list of microorganisms that affect the pictorial substrates. He also noted the inability of traditional techniques to distinguish active from inactive microorganisms, with respect to provoking alterations in artistic materials. Ciferri recommended experimental protocols for assaying the variables that appear in these types of alterations, and to establish a standardization of the variables. Moreover, he proposed the monitoring and evaluation of the initiation and rate of progress of microbial colonization, as well as changes that occur, according to substrate composition, environmental conditions, and the best way to proceed for disinfestation.

To identify microorganisms that provoke biodeterioration, Seves et al. (2000) employed traditional techniques, and also the ones of molecular biology, such as extraction of DNA, Polymerase Chain Reaction (PCR), Temperature Gradient Gel Electrophoresis (TGGE), and analysis of sequence of 16S rRNA (Helms, Martiny, Hofman-Bang, Ahring, & Kilstrup, 2004; Möhlenhoff, Müller, Gorbushina, & Petersen, 2001; Schabereiter-Gurtner, Piñar, Lubitz, & Rolleke, 2001). In a review on the microbiota and the search of new strategies for biodeterioration analysis, González and Saiz (2005) researched the diversity of the microbiota, designing different strategies and highlighting the importance of the methods to gain detection and molecular identification of microorganisms. Sterflinger (2010) reviewed the activity of the microorganisms in museums and storage facilities, that use different systems of climate control, preventive conservation, and also reviewed new systems for identification using molecular biology techniques. However, more studies are needed in this specific field of conservation.

Microorganisms exhibit different spore germination characteristics that are dependent upon substrate composition and environmental conditions (López et al., 2013). The cited study described an analytical protocol for identification of the microorganisms using molecular biology tools. The protocol included both culture-dependent and culture-independent techniques. Many microorganisms cannot be distinguished by traditional, culture-dependent techniques. Menguiano,

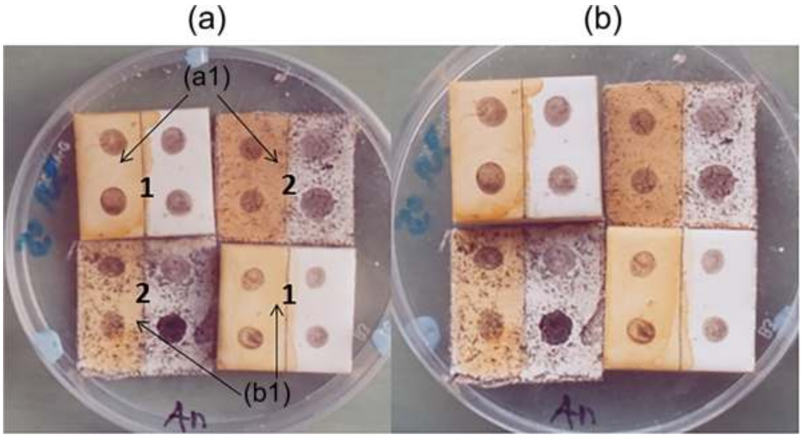

FIGURE 5 Experimental study with *Aspergillus níger* and artistic materials: 1. Wood support, 2. Textile support. White preparation layers of calcium sulfate and animal glue overlapping the support, with: (a1) almaciga varnish and (b1) colophony varnish. (a) Dish after 2 weeks of incubation. (b) Dish after 4 weeks of incubation (Poyatos, 2007)

Pérez, and Sameño (2013) instead employed culture-independent molecular biology techniques, with the innovation of cloning in cases where there was excess superposition of sequences due to high microbial diversity. The methodology applied by Piñar et al. (2015) is a key example of how to proceed in identification of species. This group analyzed the alterations that appear in the cellulose, employing SEM imaging and different techniques of molecular identification, such as Denaturing Gradient Gel Electrophoresis (DGGE), for the phylogenetic characterization of the microbial community.

Given the inherent biodiversity challenges that are posed in the identification of all microbial species in a sample (Sterflinger & Piñar, 2013), there is an additional need to i) study the physiological activity of the microorganisms in different materials; ii) carry out in-depth monitoring of antimicrobial treatments; and iii) provide alternatives involving non-toxic methods, while being aware of the impact of climatization. Therefore, we need more research in preventive conservation, with new studies on materials and biocide treatments, and developing alternative physical methods of analysis.

1.7 | Biocide treatments

The lack of specific treatments of biodeterioration of canvas paintings suggested the need to refer to studies carried out in parallel fields of artistic heritage, including conservation of stone, wood sculptures, or archives. Biocide treatments are usually employed in collections, museums, archives, storages, and buildings with artworks from the historical-artistic heritage. Currently, museums are expected to have all the guaranties in order to avoid degradative processes in their exhibits and stored materials.

Occasionally, the occurrence of microorganisms on canvas paintings does not originate from the museum deposits, in that many artworks are donations. In these artworks, restoration treatments have been carried out with organic materials added to the original constitution of the piece, such as treatment of textile support reinforcements with flour glue. Occasionally, when a lining is removed from the reverse, spots and superficial colorings in the original textile support are observed, as well as colonies and microbial structures. Thus, pieces on loan could contaminate existing items in the museum collection. It is therefore strongly recommended to diagnose the conservation condition of artwork that comes to the museum for the first time.

Initial studies on biocides addressed generic aspects of treatments for the control of fungi that grow in cultural goods. These studies also developed guidelines for conservation that should be met by buildings with the collections, as well as the treatment and preventive care that should be considered in order to minimize the growth of micro-organisms (Heim, Flieden, & Nicot, 1969). A subsequent study proposed the proper substances that should be employed in order to eradicate microbial attack of different materials, and specified the substances needed for each material (Paulus & Genth, 1990). With respect to environmental monitoring for microbial contamination that affects organic agglutinants, we highlight the study that determined the degradation provoked by fungi *Penicillium*, *Alternaria*, *Aspergillus*, *Mucor*, *Rhizopus*, *Fusarium*, *Cladosporium*, *Stemphylium*, and several species of yeast. The results of the action of these microorganisms are shown by the decoloration of pigments, loss of brightness, specific coloration, and degradation of vegetal and animal glues. Two methods for the prevention of the growth of these microorganisms were proposed. In one approach, the artwork should be maintained in environments with low humidity, and with temperature control. In the other approach, the artwork should be treated with fungicide or bactericidal agents. The methodologies were employed in cultures with isolated samples of easel paintings (Montes, 1994).

The monitoring of infestations in cultural goods was studied by Valentín (1994), and Valentín, Vaillant, and Guerrero (1996) with proposals of eradication systems for microbiota and employment of biocide treatments, focusing on museum collections and materials of archives and libraries. Also as a control of biological degradation, Caneva et al. (1996) provided ideas of protocols of action, although their work was applied to stony materials. A separate study proposed a list of applied substances toward specific microorganisms in liturgical textile material (Evans, 1996). Guillaume-Chavannes (1989) identified the properties required for the development of an ideal fungicide, such as not producing secondary harmful compounds, and having an effective long-term action. Curative and preventive methods of action were applied to disinfect polychrome sculptures, including gamma radiation, fungicide spraying (Gérard, 1998), and other treatments (Bougrain, 1998).

By the end of the 18th century, it was well known that paintings exposed to high humidity were colonized by mold (Nicolaus, 1999). As preventive remedies, oil and resin substances were applied in order to reduce the sensitivity to humidity. As an example, when paintings needed to be in spaces with high humidity, an adhesive mixture of boiled colophony and linseed oil was applied, or Venice turpentine added. *Alternaria* was cited as a source of serious damage in wood sculptures (Sameño & Rubio, 1998). This study presented methods of biological control and biocide treatments that should be employed, depending upon the material and climatic conditions of the artwork. Gérard (1998) exposed the problems of application of biocide treatments in the disinfection of diverse types of polychromies, as well as the difficulties in eliminating microorganisms without damaging the pictorial layers. Valentín and García (1999) described fungicides and their properties, regarding the elimination of microorganisms, the integrity of the component artwork materials, the application mode, and toxicity. They recommended new methods of passive ventilation as an alternative to the traditional systems employed in archives and museums.

Regarding antimicrobial treatments, there is a limited range of physical and chemical methods. An interesting approach using physical methods studied biocide systems and the employment of the inert gases helium, nitrogen, and argon as biocide substances (Koestler, 2002). Chemical methods, described by Doménech et al. (2006), employ Biotin, New-Des, and Nipagine as biocides in test tubes with varnish and several microorganisms.

The evolution of the growth of microorganisms in different artistic materials was analyzed by Poyatos (2007) with varnish of almaciga and colophony in wood and textile supports rich in cellulose. He observed that a textile support is more sensitive to microorganisms than a wood support (Figure 5). It should be mentioned that these assays are appropriate for applying biocides, and studying their effects in the materials without affecting the real artwork for the first time. There is a large list of chemical biocides described by Sterflinger and Piñar (2013); however, many of them are highly toxic.

In conclusion, the choice of the method selected is critical for successfully treating contaminated artwork. The challenge resides in the diversity of organic and inorganic materials in canvas paintings. The effectiveness of treatment should take into account: i) minimal alteration to the material constitution; ii) protecting aesthetic features; iii) selective elimination of the microbial agent that is degrading the artwork; and iv) the toxicity of the biocide treatment. These elements are not easy to satisfy to gain a successful treatment. Therefore, more research is needed in this field, with increased experimental studies on artistic materials of reference, and with the help of tools of molecular biology.

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