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**Back to the past: “find the guilty bug—microorganisms involved in the biodeterioration of archeological and historical artifacts”**

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(Article begins on next page)

# **BACK TO THE PAST. "FIND THE GUILTY BUG: MICROORGANISMS INVOLVED IN THE BIODETERIORATION OF ARCHEOLOGICAL AND HISTORICAL ARTEFACTS"**

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***ABSTRACT***

Microbial deterioration accounts for a significant percentage of the degradation processes that occur on archeological/historical objects and artworks, and identifying the causative agents of such a phenomenon should therefore be a priority, in consideration of the need to conserve these important cultural heritage items. Diverse microbiological approaches, such as microscopic evaluations, cultural methods, metabolic- and DNA-based techniques, as well as a combination of the aforementioned methods, have been employed to characterize the bacterial, archeal and fungal communities that colonize art-objects. The purpose of the present review article is to report the interactions occurring between the microorganisms and nutrients that are present in stones, bones, wood, paper, films, paintings and modern art specimens (namely, collagen, cellulose, gelatin, albumin, lipids and hydrocarbons). Some examples, which underline that a good knowledge of these interactions is essential to obtain an in depth understanding of the factors that favor colonization, are reported. These data can be exploited both to prevent damage, and to obtain information on historical aspects that can be decrypted through the study of microbial population successions.

**Key-words:** stone deterioration, syntrophic chains, wood decay, motion picture and photographic film degradation, xenobiotic degraders, amino acid racemization

## INTRODUCTION

Although exposure to physico-chemical agents can be responsible for the significant deterioration of objects of historical interest (especially outdoor objects), microbial degradation also plays a major role, due to the huge metabolic diversity of microbes and the high efficiency of the enzymes selected during evolution to ensure microbial survival in different environments. Most degradation pathways that occur on cultural heritage items are used by microorganisms for nutrition. On the other hand, metabolic products such as acids, solvents, surfactants, pigments and biofilms contribute to alter and damage artworks and archeological specimens.

Damage is sometimes caused by a predominant microbial group (e.g. the cellulolytic organisms involved in paper degradation). However, most of the time, there is a syntrophic chain in which several species contribute to a single sequenced deterioration step by releasing catabolites that become nutrients for further colonization. Identifying the microbial species involved in these complex deterioration phenomena is an essential pre-requisite for setting up rational prevention, conservation, *in situ* protection and restoration strategies. The most relevant literature data concerning the identification of the microbial species involved in the bio-deterioration of different substrates and artworks have been reported in the present mini-review.

### *Biodeterioration of stone, metal and glass material: the contribution of autotrophic organisms and syntrophic chains*

Outdoor stone monuments, caves and crypts all suffer from microbial biodeterioration. According to Martino (2016), stone material suffers from three types of deterioration: 1) esthetic surface damage, such as biofilm or pigment production 2) chemical damage, such as acid production or microbial-induced salt crystallization, which can cause discoloration and erosion 3) structural damage due to the penetration of fungal hyphae into stone. The latter, apart from causing swelling, favors water and nutrient transport inside the stone, thus leading to further bacterial colonization (McNamara and Mitchell 2005). Laiz and co-workers (2003) monitored the bacterial colonization of stone monuments and found that culturing led to an overestimation of spore-forming bacteria, and pointed out that culture-independent methods should therefore be preferred.

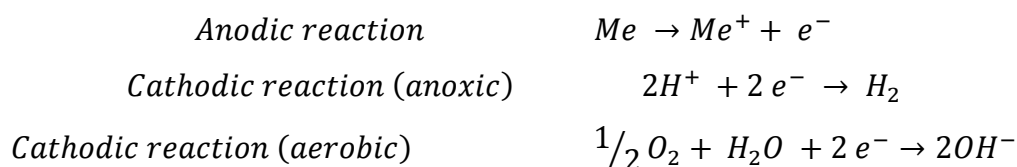
Autotrophic organisms may be the starters of syntrophic chains. Chemoautotrophs (such as the acid-producing sulfur-oxidizing and nitrifying bacteria that dissolve the alkaline material of stone) can be involved, but photoautotrophs, such as cyanobacteria, are better adapted to the oligotrophic and dry stone habitat. The latter can use  $K^+$  and  $Ca^{2+}$  ions from the rock as nutrients. Halophylic archaea, such as *Halobacterium* and *Halococcus*, can grow in salty environments. They can grow, for

instance, on stone material which shows intrinsic or biologically-driven salt efflorescences. Moreover, they can sometimes produce pink pigments, such those observed in the Johannes chapel in Pürgg (Austria) (Ettenauer et al. 2014). Because of their adaptability to even low light intensity (Kehoe and Grossman 1994), Cyanobacteria (e.g. *Fisherella*, *Eucapsis*, *Leptolyngbya*) can colonize semi-dark environments like catacombs and the *Domus Aurea* hypogeal sites in Rome (Bellezza et al. 2003), as well as outdoor monuments such as the Propylaea columns in the Acropolis in Athens (Lamprinou et al. 2013). Cyanobacteria can penetrate into a stone and create small cavities that favor water retention, thus allowing the less desiccation-resistant algae to grow (Martino 2016). Algae are frequently involved in green pigmentations, regardless of the humidity of the site, when intense light is available (Cutler et al. 2013). Once Archaea, cyanobacteria and algae growth has become established, heterotrophic bacteria and fungi can appear. Autotrophs can in fact release extracellular organic matter (i.e. biofilm), which, together with dirty abiotic particles, dust, pollen, leaves, bird excrements, mineral elements of the stone itself and dead cell, can support the growth of nutritionally exigent heterotrophs. Phototroph/heterotroph mixed species biofilms, which are frequent on stone surfaces, chemically modify a microhabitat through interspecies interactions, and this leads to reciprocal nutrition and cross-feeding, thus gaining survival for the whole community in a very harsh environment (Villa et al. 2015).

Heterotrophic bacteria, such as *Sarcina*, *Micrococcus*, *Staphylococcus*, *Bacillus*, *Alcaligenes*, *Pseudomonas*, *Flavobacterium*, *Mycobacterium* and *Nocardia* can colonize stone monuments, but the predominant action is due to the filamentous *Actinobacteria*, which can utilize a wide range of carbon and nitrogen sources (Saarela et al. 2004). *Actinobacteria* of the *Geodermatophilaceae* family (especially *Blastococcus* and *Modestobacter*, which are well-adapted to light-induced oxygen stress) have been found in arid environments (Gtari et al. 2012), such as on stone monuments in the Egyptian and Tunisian deserts. They have been characterized and clustered by means of esterase profiling (Essoussi et al. 2010). The role of both epilithic and endolithic bacteria has been reviewed extensively by McNamara and Mitchell (2005). Mycelium bearing-fungi, such as *Alternaria*, *Aureobasidium*, *Cladosporium* and *Phoma*, prevail in humid environments, whereas small-colonies black fungi belonging to *Sarcinomyces*, *Coniosporium*, *Hortea*, *Knufia*, *Exophiala*, *Trimmatostroma* and *Capnobotryella*, are more frequently isolated in dry samples (granite, marble and limestone), and sometimes in association with lichens (Sterflinger 2010). The latter are better adapted to temperature and humidity variations and thus prevail in extreme habitats (Martino 2016). A very interesting study by Cappitelli et al. showed the presence of both green-black crusts and sulfatation in different areas of Milan Cathedral (Cappitelli et al. 2007). Culture-based investigations revealed the presence of both heterotrophic bacteria (average  $10^6$  CFU/g) and fungi (average  $10^4$  CFU/g), but the use of a molecular

approach (Fluorescence in situ hybridization, FISH) also detected Cyanobacteria and Archaea. This highly innovative method exploited adhesive tape strips for the sampling, thus also providing information on the spatial distribution of the different microbial genera, without altering or damaging the stone surface.

As far as microbial induced damage of metal antiquities (e.g. coins, weaponry and statues) is concerned, corrosion may result from chemical or biochemical redox reactions. Metal corrosion can be schematized as being composed of an anodic reaction, in which the metal is oxidized, and a cathodic reaction, in which another chemical species (generally  $H^+$  or  $O_2$ , depending on whether there are anoxic or aerobic conditions) is reduced:



Microorganisms can promote metal corrosion by accelerating an anodic or cathodic reaction, or even both (Videla and Herrera 2005). Microorganisms that consume  $H_2$  generally enhance a cathodic reaction, whereas those producing acidic metabolites and/or secreting enzymes may accelerate metal oxidation (Kip and van Veen 2015). In the case of iron or steel artifacts, sulfate-reducing/sulfur-oxidizing bacteria, iron-oxidizing/iron-reducing bacteria and manganese oxidizers can act as corrosion agents (Kip and van Veen 2015). As for other inorganic materials, such as stone artifacts, metal bio-corrosion is generally the result of the activity of multi-species microbial communities embedded in biofilms (Videla and Herrera 2005). In these syntrophic chains, the role of heterotrophic species, such as *Clostridium* sp. or *Penicillium* sp. in metal corrosion cannot be neglected, since their metabolic products include both organic and inorganic acids, both of which can oxidize metals (Kip and van Veen 2015). Furthermore, the extracellular polymeric substances (e.g. exopolysaccharides, proteins, lipids) that constitute the matrix of biofilms are responsible for the metal/environment interface characteristics and can affect the electrochemical corrosion process to a great extent (Beech and Sunner 2004). The recent application of metagenomics techniques to study metal corrosion has in fact indicated that the microbial communities involved in this phenomenon are much more complex than previously thought (Marty et al. 2014; Oliveira et al. 2011). Furthermore, these researches have suggested that sulfate-reducing bacteria may not always be the main players in the bio-corrosion of metals.

Microbial-induced corrosion mainly concerns buried, sunk or poorly conserved metallic antiquities or artworks (Del Junco et al. 1992). Uncommon corrosion products, such as Mackinawite (FeS) or Greigite ( $Fe_3S_4$ ), which are ascribable to the activity of sulfate-reducing bacteria, have been

122 detected for instance on archaeological iron items, such as Roman iron ingots and nails (Rémazeilles  
 123 et al. 2010a; b). Archaeological copper artifacts and copper alloys (e.g. bronze) are also susceptible  
 124 to the metabolic activity of sulfate-reducing bacteria (Ghiara et al. 2018). Evidence of microbial  
 125 induced corrosion was found in tin-bronze decorative artifacts, greaves and swords dating back to  
 126 between the 15<sup>th</sup> and 11<sup>th</sup> century B.C., which were found in different contexts in Austria, Bosnia and  
 127 Croatia (Piccardo et al. 2013). As the result of the microbial induced corrosion of copper, a very  
 128 resistant black patina, which is rich in sulfur, copper oxides, carbonates and/or hydroxy-chlorides, is  
 129 formed (Ghiara et al. 2018).

130 The study by Marvasi et al. (2009) has shown that the bacterial colonization of medieval  
 131 stained glass windows in Florence cathedral was favored by dust, crusts and organic matter. The  
 132 inside of the windows did not exhibit any visible damage, whereas the outside of the glass was clearly  
 133 contaminated with crusts, except for the green parts of the windows where no damage was detected,  
 134 even on the external parts. Hence, the authors analyzed the chemical composition of the green glass,  
 135 hypothesizing an antibacterial activity of the glass component(s). Copper (present in high quantities  
 136 in green glass), which in its Cu<sup>2+</sup> form is toxic for microbial cells, and has been demonstrated to  
 137 reduce colonizer biodiversity (Milanesi et al. 2006), was not the cause of the lower number of  
 138 microorganisms that were found, since most of the bacteria were resistant to CuSO<sub>4</sub>. Similarly, lead  
 139 (Pb) was not involved, since it was only present on the internal side of the glass. However, a higher  
 140 Na content was found in the green glass than in the other colored glass. The Na-rich glass also  
 141 displayed a higher silica content (around 65%) than the K-rich glass. In particular, the green glass  
 142 was found to be of the so-called Na-rich alkaline silicate-sodium type of glass that is typical of the  
 143 medieval Renaissance period. This condition is unfavorable for microbial growth. Conversely, the K-  
 144 Ca-SO<sub>4</sub> crusts found on the other colored glass (ascribable to gypsum and syngenite, as determined  
 145 by means of Fourier Transform Infrared Spectroscopy, FTIR) created a microenvironment that was  
 146 able to retain nutrients, microorganism and moisture, which in turn protected the bacteria from the  
 147 high temperatures reached as a result of exposure to sunlight during the day. The combination of  
 148 microscopic/biochemical identification with 16S rDNA-based molecular tools indicated that the  
 149 populations found inside the cathedral were different from those found on the outside glass;  
 150 *Firmicutes* in particular were absent on the inside windows. The clean glass (inside and green) mainly  
 151 hosted *Actinobacteria* and *Proteobacteria*. The former were previously also found on other Cathedral  
 152 windows (Krumbein et al. 1991; Rölleke et al. 1999), whereas the proteobacteria *Brevundimonas* are  
 153 typical of alkaline and nutrient-poor environments (Abraham et al. 1999). A high number of spore-  
 154 forming *Bacillus* and *Paenibacillus* were found in the crusts, thus indicating that the sporulation  
 155 ability could have been responsible for their resistance and long survival ability, as reported above.

## ***Wood and paper biodeterioration: the role of lignocellulolytic microorganisms***

Lignocellulosic material is the main component of paper, vegetal textiles and wood. Lignocellulose mainly includes cellulose, hemicellulose and lignin, whose relative amounts may widely vary depending on the specific item (Bomble et al. 2017). It is an energy/carbon substrate for many different microorganisms, including both bacteria and fungi. These organisms may damage library book collections, ancient documents, drawings and photographs (Cappitelli et al. 2010), as well as wooden objects, e.g. ancient coffins, weapons, Native American houses, boats, bridges, ships and shipwrecks (Björdal 2012a and 2012b; Björdal et al. 1999; Palla et al. 2013; Singh 2012). Lignocellulose deconstruction in the biosphere is a complex phenomenon which is generally catalyzed by mixed microbial communities, in which each strain provides its peculiar enzyme activity(ies) (e.g. lignin and/or cellulose and/or hemicellulose depolymerizing action) (Bomble et al. 2017). The best characterized lignin degraders are white-rot and brown-rot fungi which use oxidative mechanisms, i.e. peroxidases and laccases or Fenton chemistry, respectively (Bomble et al. 2017). Hemi-/cellulolytic microorganisms mainly biosynthesize glycoside hydrolases and polysaccharide lyases, although other biochemical mechanisms for hemi/cellulose depolymerization have been recently discovered (Bomble et al. 2017). Cellulolytic organisms are also involved in the deterioration of fabric of vegetal origin. However, this aspect will be treated in more detail in the next section (textile deterioration).

### **Wood**

Unlike what occurs for above-ground wood, whose decay is mainly due to strictly aerobic fungi (e.g. Basidiomycetes, such as white-rot and brown-rot fungi) and is very fast (less than one year- a few years) (Daniel and Nilsson 1997), buried or waterlogged wood is prevalently degraded by moderate aerobic and anaerobic organisms (soft rot Ascomycetes and Deuteromycetes, tunneling bacteria and erosion bacteria) (Singh 2012). In the latter case, the degradation occurs at a much lower rate (hundreds, sometimes thousand of years) (Björdal 2012a). Lignocellulose degradation is much faster on land (provided that wood is in contact with the ground) than in aquatic environments, because of the greater oxygen availability, which also accounts for lignin degradation. In underwater sites, such as peatlands, seas and lakes, only water-dissolved oxygen is available, thus microaerophilic and anaerobic organisms prevail. Since their metabolism is slower, decay takes longer. It is for this reason that important archeological wood samples, especially ships, have been preserved until now. For example, the Vasa warship and the Oseberg Viking ship (Fig. 1) have both been preserved by an aquatic environment in which a “low profile degradation” occurs (Björdal 2012a). However, in spite of the apparent good state of preservation (physical integrity, presence of colors, ornaments etc.)



189 archeological wood behaves very differently from recent sound wood: it is spongy and very soft, and  
 190 if it is not kept wet, it will crack and disintegrate (Björdal 2012a).

191 The first attempt to identify microbial communities on waterlogged archeological wood was  
 192 reported by Björdal and co-workers in 1999. These authors demonstrated, by means of SEM, that  
 193 anoxic-tolerant erosion bacteria (EB) can be found throughout wood tissue, whereas a prevalence of  
 194 tunneling bacteria (TB) and soft root fungi (SR, Ascomycetes) can be observed in the outer layers.  
 195 EB attack was also monitored by means of polarization light microscopy and transmission electron  
 196 microscopy (TEM), and the results demonstrated cellulose depletion and lignified cell-walls with  
 197 typical crescent-shaped grooves (Singh 2012). Although the authors did not identify the bacteria,  
 198 these studies had the merit of demonstrating that biological deterioration was the main reason for  
 199 wood damage in this extreme environment. The environment was in fact slightly alkaline, and the  
 200 presence of bacteria was determined microscopically. Furthermore, these authors attempted to  
 201 establish the location of the ship waterline (by means of microscopic observations), which could  
 202 constitute an important parameter to help estimate the ship weight and hence the type of material  
 203 transported. The study compared the microbial populations of two waterlogged archeological ships.  
 204 In the former, found in the site named Kronholmen (Sweden), the typical decay of EB was observed,  
 205 thus suggesting a very early sinking of the ship, which favored anaerobic degradation. On the other  
 206 hand, an abundance of SR fungi attack was reported for the latter ship found in Kraveln (Sweden),  
 207 thus indicating that the decay probably occurred when the ship was still sailing. Finally, a medieval  
 208 house found in the terrestrial medieval layers of the Vadstena site (Sweden) displayed the particular  
 209 signature of brown rot fungi degradation (Björdal et al. 1999). Since these organisms are even more  
 210 aerobic than SR, this finding suggests that the house was colonized by decay-microorganisms when  
 211 it was still in use. These studies have all had a great significance for archeologists.

212 It should be underlined that, apart from oxygen (lower oxygen, lower decay), other factors  
 213 (such as soil type, salinity, pH and temperature) also account for a faster or slower degradation, and  
 214 favor certain microbial populations (Björdal 2012a). For instance, salty waters favor wood  
 215 degradation by marine borers even earlier than microbial intervention. High nitrogen availability  
 216 favors SR fungi, while EB seem more adapted to low nitrogen concentrations (lower than 0.1%) and  
 217 TB are selected in a relative alkaline environment (Björdal 2012b). Finally, the susceptibility of each  
 218 type of wood is crucial as is the wood species. In general, type1 SR prevalently colonize  
 219 gymnosperms, whereas type 2 SR colonize angiosperms (Singh 2012). Moreover, oak and pine  
 220 display a higher resistance to decay than birch (Björdal 2012a).

221 In 2004, Helms and coworkers analyzed anaerobic bacteria that colonized an ancient wooden  
 222 spear shaft, which was found in an archaeological site in southern Jutland (Denmark), by extracting

and amplifying 16S rDNA sequences from the individual cultures after growth on glucose and xylose at 14°C and 20 °C, and they found clones belonging to alpha, beta and delta proteobacteria. Nilsson et al. (2008) have recently characterized the microbial populations of EB on archeological waterlogged wood using DNA-based techniques, while referring to the ribosomal RNA clone libraries and DGGE set up by Landy et al. (2008). Although most of the bacteria belonged to the *Cytophaga-Flavobacteria* cluster, the identification of these bacteria at a species level has still not been achieved. A review article that reported on the biodegradation phenomena that occurs on underwater wrecks in the Baltic Sea (Björdal 2012b) describes how true wood degraders (e.g. microorganisms that are able to directly depolymerize lignin and/or cellulose and/or hemicellulose) generally coexist with bacteria (which are also responsible for iron and sulfur cycling) that are able to use the soluble sugars, such as mono- and oligo-saccharides, or end-products (e.g. lactic acid, acetic acid, ethanol) derived from lignocellulolytic species metabolism. This points out the important synergistic interactions that occur among different underwater wood inhabitants.

A combined approach (SEM, bacterial cultures and DNA-based techniques) was used by Palla et al. (2013) to characterize the bacterial population of an underwater fleet wreck (36 B.C.) in the Sicilian area. Amplification of specific ribosomal DNA sequences, like the Internal Transcribed Spacer (ITS), allowed *Xanthomonas*, *Pseudomonas*, *Sphingomonas* and *Marinobacter spp* to be identified.

#### Paper

As far as paper documents are concerned, the work by Cappitelli et al. (2010) led to the identification of cellulolytic microorganisms on an ancient Italian manuscript (dating back to 1293 A.D.) as well as on the Leonardo da Vinci Atlantic Code (early years of 1500 A.D.). For the latter, the authors developed a non-invasive sampling procedure with sterile nitrocellulose membrane filters and used them for direct DNA extraction. This DNA was studied by means of Denaturing Gradient Gel Electrophoresis (DGGE) (Fig. 2a) of the 16S rRNA and ITS regions, and this allowed band patterns to be analyzed by the principal component analysis (PCA) multivariate technique. The construction of bacterial and fungal clone libraries is useful in the detection of true degraders among different organisms (for instance, skin microbiota contaminants, insect-carried bacteria) and to reveal the microorganisms that possess the endo- or exo-glucanases that are able to depolymerize cellulose. Cellulolytic activities can also be detected using cellulose powder mixed in an agar medium and then observing whether a clear halo appears in the agar plate (Cappitelli et al. 2010). Electronic nose technology can also help in discriminating volatile acids produced by the cellulolytic activities of *Aspergillus* and *Eurotium* (Canhoto et al. 2004).

In short, the analyses of ancient paper have highlighted that microbial colonization occurs mainly when the relative humidity is above 65% and the temperature is higher than 23°C, as this facilitates the growth of several fungal genera (*Alternaria*, *Aspergillus*, *Mucor*, *Penicillium*, *Rhizopus*, *Cladosporium*, *Chrisosporium* and *Trichoderma*) as well as cellulolytic bacteria. It should be pointed out that modern paper is different from ancient paper: in the former, apart from cellulose, other components of wood pulp, such as hemicellulose, pectin and lignin, can represent a suitable carbon substrate for microbial colonization. Furthermore, modern paper documents are treated with gelatin and pigments to confer additional properties, thus constituting a supplementary source of nutrients for microbial colonization (Cappitelli et al. 2010).

***Textile material biodeterioration: cellulolytic, keratinolytic and esterase-producing microorganisms.***

Archeological fabrics (Native Indian clothes, Pre-Columbian and Egyptian textiles, soldiers' uniforms, ecclesiastical vestments, shrouds, carpets, tapestries, oil-on-cotton paintings) are precious items that generally reveal a poor conservation quality. Microbial growth on textiles can produce unwanted pigmentation (e.g. blue or brown spots) discoloration, the presence of biofilms, but also the loss of strength, a decrease in elasticity, depolymerization, disruption of the fiber structure with textile cracking and fragmentation, all of which creates damage that needs to be repaired, and the presence of the microorganisms that are responsible has to be ascertained.

Textiles constitute a nutrient rich environment that can support the growth of both bacteria and fungi. Clothes such as nurses' uniforms can even act as a reservoir for multidrug resistant bacteria (Neely and Maley 2000). The intrinsic nature of a textile is crucial in favoring or preventing colonization. Because of their hydrophilic structure, natural fabrics retain humidity and thus provide a perfect habitat for microbial colonization. On the other hand, synthetic hydrophobic fibers are more recalcitrant to biodegradation. External factors, such as high relative humidity, light exposure, high temperature, spontaneous oxidation and aging, can also be responsible for inducing a faster degradation (Szostak-Kotowa 2004). However, degradation is just as likely in dark sites, such as tombs, graves and crypts, because of the high water content (Gutarowska et al. 2017).

As far as pigmentation is concerned, tents, sails and beach umbrellas, being exposed to sunlight and humidity, can support the growth of algae that generate green pigments, whereas raw wool (fleece) can be colonized by *Pseudomonas aeruginosa*, which generates both green (in an alkaline environment) and red (in acidic conditions) pigments during wool degradation. Yellow, orange, brown or black pigments can also be synthesized by *Brevibacterium*, *Bacillus*, *Rhodococcus*, *Corynebacterium*, *Achromobacter*, *Streptomyces*, and by fungi such as *Rhodotorula*, *Penicillium*, *Aspergillus*, *Cryptococcus* (Gutarowska et al. 2017). Discoloration is often the consequence of an

290 altered pH, due to microbial metabolism. Culturing is not a suitable method for detecting the “guilty  
 291 microbes” since about 99% of microbial strains are viable and metabolically active, but not culturable.  
 292 Hence, culture-independent methods can be applied successfully to solve the problem. Techniques  
 293 based on the amplification of target/marker genes (e.g. 16S rRNA in bacteria and 18S rRNA in fungi),  
 294 followed by different approaches, such as DGGE, ARDRA (Amplified Ribosomal DNA Restriction  
 295 Analysis), SSCP (Single strand conformation polymorphism), ARISA (Automated method of  
 296 ribosomal intergenic spacer analysis) and NGS (Next Generation Sequencing), have been used to  
 297 characterize microbial populations at the species level (Lech et al. 2015). However, culturing methods  
 298 followed by molecular-based microbial identification have recently also been used by Pietrzak and  
 299 co-workers (2017) to identify microbial populations on Pre-Columbian Textiles made of cotton and  
 300 lama- or alpaca-wool. Bacteria from the *Bacillus*, *Oceanobacillus*, *Staphylococcus*, *Micrococcus*,  
 301 *Pseudomonas* genera strains were isolated as well as more abundant quantities of *Kokuria rosea* and  
 302 *Paracoccus yeei*. The most common fungal genera were *Aspergillus*, *Penicillium* and *Cladosporium*.  
 303 The authors underlined that a greater biodiversity can be present on cotton samples, with 11 different  
 304 species having been isolated (Pietrzak et al. 2017).

305         Vegetal and animal fibers display different resistance to biodegradation (the former being  
 306 more sensitive than the latter), and they hence have different destinies: some microbial degradative  
 307 pathways will be described in the following sections. However, it should be pointed out that  
 308 susceptibility to biodeterioration is also related to the type of weave, the textile thickness and the  
 309 polymerization extent of the fiber, as well as to its amorphous or crystalline state.

#### 310         *Cotton, linen, jute and hemp*

311         Plant-derived fabrics are susceptible to the action of lignocellulolytic enzymes. Non-cellulosic  
 312 components, like lignin, render the fiber more resistant to degradation: for example, hemp and jute,  
 313 which contain a high percentage of lignin, degrade more slowly than cotton, which lacks such  
 314 compounds (Gutarowska et al. 2017). Conversely, pectin and hemicellulose are easily degradable and  
 315 favor microbial colonization, which in turn promotes the attack of cellulolytic organisms (Szostak-  
 316 Kotowa 2004). Three types of hydrolytic enzymes are required for complete conversion of cellulose  
 317 into glucose (the true energy-generating carbon substrate): 1) *exoglucanases*, which cleave cellulose  
 318 chains, starting from the reducing or non-reducing end, and generate cellobiose or glucose 2)  
 319 *endoglucanases*, which cleave internal glycosidic bonds of amorphous cellulose in a random manner  
 320 and generate different-length oligosaccharides 3) *beta-glucosidases*, which convert short  
 321 oligosaccharides, such as cellotriose and cellobiose, to glucose. Generally, bacteria such as  
 322 *Cellulomonas*, *Cellvibrio*, *Clostridium*, *Cytophaga*, *Bacillus*, *Arthrobacter*, *Sporocytophaga*,  
 323 *Microbispora*, *Pseudomonas*, *Nocardia* and *Streptomyces* act from the fiber surface toward the

interior. Conversely, most fungi (*Aspergillus*, *Verticillium*, *Penicillium*, *Mucor*, *Myrothecium*, *Thricoderma*, *Rhizopus*, *Alternaria*, *Fusarium*, *Aureobasidium* and *Cladosporium*), or their spores, penetrate directly into the fiber lumen, where they generate a mycelium that is responsible for the secretion of extracellular cellulolytic enzymes (Szostak-Kotowa 2004). The final effect of cellulase action is the depolymerization of cellulose, which leads to an impaired fiber strength.

#### *Wool and silk*

Animal-derived fabrics are a little more resistant to degradation. Their main components are proteins: keratin in wool and fibroin and sericin in silk, hence, proteases are required for degradation.

Keratin is a compact structure made up of parallel or antiparallel peptide chains, cross-linked by disulfide bridges. This is why hair and wool are long lasting post-mortem. However, insects can attack wool keratins, as well as bacteria and fungi. Keratinolytic bacteria (*Alcaligenes*, *Bacillus*, *Proteus*, *Pseudomonas* and *Streptomyces*) are less efficient than fungi. Among the latter, *Fusarium*, *Rhizopus*, *Aspergillus*, *Penicillium*, *Microsporum*, *Chaetomium*, *Trichophyton* and *Trichoderma* have been described as significant keratin degraders. The degradative action begins with a reduction of the disulfide bridges, which results in a weaker polypeptide chain that is suitable for proteolytic attack (Szostak-Kotowa 2004). Peptide degradation can also give rise to ammonia as a result of amino acid deamination (Gutarowska et al. 2017).

As far as silk is concerned, its main protein fibroin is made up of fibers held together by sericin, a second protein that acts as an adhesive. While fibroin is essentially constituted (more than 90%) by four amino acid repeats (glycine, alanine, serine and tyrosine) that are less attractive as microbial food, sericin is the first one to be utilized as a nutrient by microorganisms. Degummed (i.e. sericin-deprived) silk is degraded at a slower rate, and two months are required before a decrease in strength can be detected. Nevertheless, sericin-deprived silk is more susceptible to light damage. Although only *Pseudomonas cepacia* can use fibroin as a carbon source, *Bacillus*, *Serratia*, *Pseudomonas* and *Streptomyces* have also been found in a degradation mixture, thus suggesting the occurrence of co-metabolization (Forlani et al. 2000; Seves et al. 1998). One fungal strain of *Aspergillus niger* has also been described as being able to modify the fibroin structure (Szostak-Kotowa 2004).

#### *Man-made textiles*

Man-made textiles may be of natural or synthetic origin. Viscose (also called rayon), a natural fiber originating from cellulose, is very sensitive to microbial degradation. Other synthetic polymers, as previously mentioned, display a certain degree of resistance, because of their hydrophobicity, but also because of their intrinsic chemical bonds (i.e. ether), which are unusual in natural compounds. Polyurethanes are not so hydrophobic, and they can therefore bind water, thus favoring microbial

colonization. Polyester-containing polyurethanes are generally degraded faster than polyether-containing polyurethanes, thus confirming the importance of the intrinsic chemical bonds (Seal 1988). Polyurethane is the most suitable polymer for microbial degradation, because it contains domains (ester bonds, urea) that mimic natural bonds. Extracellular fungal esterases may catalyze polyurethane degradation: *Alternaria*, *Aspergillus*, *Penicillium*, *Trochoderma* and *Cladosporium* are among the fungal genera involved in this process. Polyurethane is often employed for the production of bathing wear, because of its elasticity and flexibility. Swimsuits from Olympic winners in museums are at risk of damage as a result of exposure, and particular care should be taken to house these items in a sterile environment (Rowe and Howard 2002).

Regardless of their intrinsic features (lesser or higher degradability), synthetic fabrics are often treated with oils, fats, pigments and plasticizers to finish the textile. These additives can contain nutrients that support microbial growth, and later favor fiber disruption and fabric deterioration. A paradigmatic example is polyvinyl chloride (PVC), which is used for waterproof coatings, and which is not a microbial nutrient in itself, but is often treated with plasticizers, such as aliphatic polyesters, to enhance elasticity. Aliphatic polyesters and lactic acid polymers (such as PLA) are easily degraded by microorganisms that can later alter PVC by co-metabolism (Webb et al. 2000). Furthermore, during use, dirty particles can accumulate, thus adding supplementary nutrients for colonization.

Polypropylene and polyamide fibers, like nylon, are generally degraded after exposure to light, since UV-induced photo-degradation accelerates the bioavailability of shorter chain polymers. Among the bacteria, *Bacillus*, *Bravibacterium*, *Achromobacter* and *Protaminobacter* can all degrade nylon after exposure to light. This should be taken into account when synthetic materials of cultural heritage interest are on display in museum areas under intense light. On the other hand, a *Pseudomonas aeruginosa* strain that is able to hydrolyze nylon without prior light exposure has been observed (Priyambada et al. 1995). As far as polyacrylonitrile (acrylic textiles) is concerned, an *Arthrobacter* strain, which can utilize acrylonitrile as a nutrient, has been isolated, but not its polymer (Seal 1988). Polyethylene terephthalate (PET), like other aromatic polyesters, seems to be, among plastic polymers, the most resistant to microbial attack (Szostak-Kotowa 2004). However, due to the intense search of xenobiotic-degrading organisms, a Gram-negative aerobic beta-proteobacterium, named *Ideonella sakaiensis*, which is able to degrade PET, was isolated two years ago (Yoshida et al. 2016). Although some constraints limit full degradation (i.e. the process is relatively slow, access to the PET polymer fibers in the smooth plastic surface is not so easy), there is good possibility that this, and possibly other bacteria, will be able to attack PET objects in the future.

***Bone deterioration: contribution of collagenase and amino acid racemization activities.***

391 Among the various animal tissues, bones are the best preserved after death. It is for this reason  
 392 that archeological bones are so important in the reconstruction of events, such as the historical life-  
 393 period of a civilization found in an excavation site, the species determination of bones of unknown  
 394 taxonomy and the cause and the age of death of human remains. However, deterioration can also  
 395 occur on bones, and microbial degradation plays a crucial role. This event occurs very early (3  
 396 months-5 years after death), depending on the humidity, temperature and the oxygen availability, and  
 397 is largely determined by endogenous gut bacteria or soil microorganisms (Jans et al. 2004). The  
 398 macroscopic alteration of bones is named “tunneling”, since empty tunnels of about 10  $\mu\text{m}$  of  
 399 diameter appear, thus indicating that both the mineral and the proteinaceous components of the bones  
 400 have been destroyed by microorganisms. A high percentage of tunneling is due to bacterial activity  
 401 (Jans et al. 2004). Bacterial degradation generally occurs on demineralized bones, since both body  
 402 fluids and soil components can create an acidic environment that favors demineralization. However,  
 403 some bacteria can directly liberate proteins from inorganic material (Child 1995a; Kendall et al.  
 404 2018).

405 Collagen is the most represented protein in bones. Although the terminal parts of collagen are  
 406 sensitive to the proteolytic action of chymotrypsin and pepsin, the helical portion of collagen is only  
 407 hydrolyzed by specific collagenases, i.e. enzyme complexes made up of six different subunits  
 408 containing zinc in the catalytic center. Because of this resistance to enzymatic degradation, collagen  
 409 is a long-lasting protein (Giuffrida et al. 2018). However, the typical bacterial collagenases of  
 410 anaerobic Clostridia (for instance, *Clostridium histolyticum*), but also of aerobic *Mycobacterium*  
 411 *tuberculosis* (Child 1995b), *Pseudomonas spp*, *Aeromonas* and *Klebsiella* (Child et al. 1993) can alter  
 412 collagen stability. Unlike what is observed in bacteria, only one fungal species (*Chrysosporium spp.*),  
 413 among those isolated from bones, displays collagenase activity, thus suggesting that soil fungi are not  
 414 the first bone colonizers (Child et al. 1993).

415 The extent of racemization of bone collagen was used in the past to determine the time that  
 416 had elapsed since the death of an individual, or to predict the preservation of DNA in the bone (Bada  
 417 and Protsch 1973; Poinar et al. 1996), but both uses have been abandoned, since the open-system  
 418 nature of bone and the structure of collagen itself prevent predictable patterns of diagenesis (Collins  
 419 et al. 2009; Demarchi and Collins 2014; Wadsworth et al. 2017). Unfortunately, some bacteria  
 420 (*Pseudomonas spp*, *Aeromonas*) can express non-specific amino acid racemases that can alter the  
 421 ratio between R and S forms, as well as preferentially metabolize one enantiomeric form (Child et al.  
 422 1993), thus making the real age at death of archeological bones questionable. Jans and co-workers  
 423 (2004) combined histology and mercury intrusion porosimetry to study archeological bones from  
 424 excavations in different geographical areas (Mediterranean, coastal, subarctic and continental). They

demonstrated that bones from the abdominal area are rapidly colonized by intestinal bacteria, such as *Clostridia*, *Staphylococci* and *E. coli*, whereas dismembered animal bones are not attacked by endogenous microflora and therefore constitute a nutrient-rich medium for soil fungi. Since most fungi are strictly aerobic, oxygen availability is a limiting factor for degradation. Therefore, a better conservation state can be observed when the burial ground has a low redox potential.

***Painting biodeteriogens: lipolytic, amylolytic, proteolytic, solventogenic, acidogenic and pigment-producing microorganisms.***

Wall and easel paintings can suffer from biodeterioration related to the degradation of the material itself (due to microbial enzymatic activities), or to the production of primary or secondary metabolites. Metabolic end-products, such as surfactants, solvents and acids, can cause the discoloration or corrosion of artefacts. Secondary metabolites, like pigments (generally produced as defense molecules), can produce stains. Since a painting can be performed on any material, the number of possible nutrients for microbial growth increases. Several layers should be considered, e.g. a support material, thickeners and glues, pigments, emulsifiers, protective films, but also unwanted exogenous particles that can carry nutrients.

Carbon sources found in wall paintings can select autotrophic bacteria, whereas easel paintings (on wood, wool, silk, paper, etc.) support the growth of heterotrophic organisms. A nitrogen source is sometimes present in the support (keratin in wool, fibroin in silk), or can be supplied by the glues (for instance, collagen-based glues) or the emulsifiers/protectants (milk was frequently used, before the acrylic era, to create a protective glossy film on paintings, thus supplying caseins). However, natural pigments (e.g. those based on egg-yolk) are the best sources of different nutrients, especially on ancient medieval paintings (Giuffrida et al. 2018). Egg-white and egg-yolk can both contribute to albumin and vitellogenin availability for microorganisms, but can also supply lipids as an energy source. In general, it is possible to state that wall paintings are more susceptible to biodeterioration than easel paintings, since they are generally conserved in rain-exposed environments or in humidity rich hypogeal sites that favor microbial colonization. For this reason, most literature data refer to frescoes.

Several alternative approaches have been employed/developed to characterize the causative agents of biodeterioration. In 1996, Rölleke and co-workers characterized the microbial population on a 13<sup>th</sup> century wall painting belonging to the Chapel of the Herberstein Castle in Austria. By means of electron microscopy, they detected bacteria that had a filamentous morphology. Culturing allowed the growth of only five strains, three of which gave rise to pigmented colonies (white, yellow and red, respectively). DGGE analysis on the amplified DNA from the purified isolates revealed the presence of *Actinomycetales* (high G+C content Gram-positive bacteria like *Arthrobacter*, *Pseudonocardia*



459 *and Streptomyces*) and *Acinetobacter lwoffii*. The former possess the ability to form hyphae that can  
 460 cause frescoes to lose their integrity through a mechanical disruption of the wall layers. The latter can  
 461 occur since many species of the same genus (Gram-negative belonging to the gamma proteobacteria)  
 462 use short-chain fatty acids and lipids as preferential carbon sources (Violetta et al. 2014). It was  
 463 probably at the expense of egg-yolk pigments or oils used as emulsifiers that these bacteria could  
 464 grow on the Chapel of the Herberstein Castle paintings. The DGGE approach was also used to study  
 465 DNA aliquots, sampled directly on the wall painting, without prior cultivation. *Halomonas*,  
 466 *Clostridium* and *Frankia* were detected. *Frankia*, an Actinomycetes that displays very slow growth,  
 467 can be responsible for mechanical damage due to hyphae, although it is seldom referred to in the  
 468 literature because it is difficult to cultivate. *Halomonas* (Gram-negative belonging to the gamma  
 469 proteobacteria) can be found in extremely salty environments (e.g. the salt efflorescence areas of  
 470 frescos) and can cause biodegradation, due to acid production, when its metabolism shifts from  
 471 aerobiosis (respiration) to anaerobiosis (fermentation). *Clostridium* (low-G+C content Gram-positive  
 472 bacteria) are obligate anaerobes that produce acids and alcohols from both carbohydrate and protein  
 473 fermentation. Some alcohols, like ethanol and butanol, can have a solvent action on pigments, thus  
 474 causing fresco discoloration. Finally, the authors highlighted the importance of using molecular  
 475 methods to ensure the right ratio among the different populations. For instance, although  
 476 *Acinetobacter* gives rise to a significant biomass, it was not so abundantly represented in the DGGE  
 477 pattern (Rölleke et al. 1996). On the other hand, the same work group found different microorganisms  
 478 using cultivation vs molecular methods, and suggested that it is necessary to combine the two  
 479 techniques in order to have a true picture of what happens on a mural painting surface (Gurtner et al.  
 480 2000).

481 Radaelli and co-workers (2004) characterized the microbial populations present on a damaged  
 482 17<sup>th</sup> century fresco in Assisi (Italy) through morphological observation and traditional biochemical  
 483 methods. They found a prevalence of Gram-positive cocci (mainly *Micrococcus* and *Staphylococcus*),  
 484 followed by Gram-negative rods (mainly *Pseudomonas* and *Alcaligenes*) and then by Gram-positive  
 485 rods (only *Corynebacterium* and *Bacillus*). The most abundant species, *Staphylococcus cohnii* and  
 486 *Bacillus licheniformis*, were submitted to molecular bio-typing to detect whether there were any intra-  
 487 species differences among the several strains that had been isolated. Restriction Fragment Length  
 488 Polymorphism (RFLP) (Fig. 2b) and Random Amplified Polymorphic DNA (RAPD) analyses both  
 489 revealed a genetic similarity of the studied strains. Considering the biodeteriogenic potential of the  
 490 different isolates, the authors proved that *Pseudomonas maltophilia* was absent in the less damaged  
 491 areas, thus suggesting its role in the degradation of the most damaged parts (Radaelli et al. 2004).

492 Fatty acid methyl ester analysis (FAME) (Fig. 2c) was used to detect the biodiversity of  
 493 bacterial strains isolated from a wall painting belonging to St Catherine's Chapel (Herbstein,  
 494 Austria) and to St. Martin's Church (Greene, Germany) (Heyrman et al. 1999). Again in this case,  
 495 Gram-positive bacteria, including *Bacillus*, *Paenibacillus*, *Arthrobacter*, *Micrococcus* and  
 496 *Staphylococcus spp.* were ubiquitous and highly represented. *Nocardioform actinomycetes* were only  
 497 found in the Greene site, whereas *Halomonas* was only found in the Herbstein site, suggesting that  
 498 particular conditions favor the presence and selection of these species. The authors explained that the  
 499 high number of *Bacillus* strains they found in samples from different geographic sites was due to the  
 500 fact that the sporulation ability makes them able to survive for long periods of time.

501 An interesting paper by Imperi et al. (2007) reported the characterization of both bacteria and  
 502 pigments detected on a 9<sup>th</sup> century fresco, illustrating scenes from the Genesis (Fig. 3). These  
 503 byzantine paintings, discovered in 1963 in the Crypt of the Original Sin near Matera (Italy), had  
 504 suffered from water infiltration, carbonate precipitation and discoloration. Former attempts to  
 505 characterize the microflora, by means of morphological and culture-based methods, had revealed the  
 506 presence of cyanobacteria and green algae. Later, an unwanted reddish pigmentation that covered  
 507 much of the painted area appeared. Background-subtracted *in situ* micro Raman spectra of the  
 508 pigmented area revealed three major bands, ascribable to the vibrational mode of the C-CH<sub>3</sub> groups,  
 509 to the single C-C bonds and the double C=C bonds, respectively. The analytical results made it  
 510 possible to conclude that the pigments were carotenoid molecules. Both ARDRA and DGGE were  
 511 used for microbial typing. Actinobacteria (in particular *Rubrobacter radiotolerans*),  $\alpha$ -Proteobacteria  
 512 (in particular *Erythrobacter spp.*), Bacteroidetes (in particular *Sphingobacterium*) and Cyanobacteria  
 513 were found to be present, as well as Archea such as *Halococcus* and *Haloferax*. However, Archea  
 514 only represented a numerically insignificant contaminant (less than 0.1% of the 16S rRNA gene pool),  
 515 whereas *Rubrobacter radiotolerans* was abundant (about 87% of the 16S rRNA gene pool per  
 516 sampled site) in almost all the samples from the pigmented area. In order to better assess the cause of  
 517 the pigmentation, pigments produced by *Rubrobacter radiotolerans* were analyzed by micro Raman  
 518 spectroscopy, and it was demonstrated that they were the same as the pigmented area on the fresco.  
 519 These carotenoids, named bacterioruberins, have a C-50 length and display 13 conjugated double  
 520 bonds. However, this result cannot exclude that other microbial strains (eubacteria, such as  
 521 *Micrococcus* and *Arthrobacter* and archea like *Halococcus* and *Haloferax*) could also synthesize  
 522 ruberins, since the Raman analysis was unable to distinguish bacterioruberins from different species.

523 *Motion picture films and photographic material biodeterioration: the contribution of*  
 524 *gelatine liquefiers.*

Cinematographic films and photographs have an important historical value. They are both composed of three basic elements, namely a *support*, an *image-forming layer* and a *binder* for the image-forming emulsion. These layers can undergo both abiotic deterioration and microbial attack. The latter can cause degradation, pigmentation and discoloration (Abrusci et al. 2005). Owing to their relative recent origin, no attention has been paid to ensuring their conservation, and it is only in the last two decades that papers dealing with this problem have begun to appear in the literature.

Until the end of the last century, the support material was made of cellulose esters, mainly cellulose nitrate (used since the end of the 19<sup>th</sup> century until 1950) and cellulose triacetate (CTA, in use between 1950 and 2000). Both are excellent growth media for cellulolytic bacteria and fungi, although the higher the esterification is, the higher the resistance to microbial degradation (Sakai et al. 1996). Since 1990, synthetic plastics, such as PET (polyethylene terephthalate), have been used to overcome the poor chemical stability of natural polymers, and these are able to guarantee a 10 times longer life-time than cellulose esters. However, microorganisms that are able to degrade PET are being described more and more frequently in the literature, and *Ideonella sakaiensis* 201-F6 has recently been included in this list (see the previous section) (Yoshida et al. 2016).

As regards support material, before undergoing cellulolytic degradation by fungi and bacteria, CTA must be de-acetylated by esterases. De-acetylation can be also obtained abiotically under suitable temperature and moisture conditions (the phenomenon that releases acetate has a characteristic odor which is referred to as a “vinegar smell”) (Abrusci et al. 2004a). When a suitable degree of de-acetylation has been obtained, and at least two adjacent glucose units are available, cellulase-mediated catalysis can occur. *Aspergillus*, *Penicillium*, *Fusarium* and *Trichoderma* have been reported as CTA degraders among fungi, while *Pseudomonas* and *Neisseria* have been reported among bacteria (Abrusci et al. 2004a).

Photosensitive emulsion includes silver salts (in black and white photographs) and pigments (in colored photographs) mixed with gelatin (an amorphous transparent material that forms a gel network, obtained by thermal denaturation of animal collagen), which constitutes the binder (Fig. 4). Although silver can be toxic for living organisms, most fungi display the ability to reduce dangerous oxidized silver ions into metallic silver, which is then accumulated as nanoparticles on the cell-wall surface (Sclocchi et al. 2013). However, the deterioration of films is very seldomly linked to the microbial utilization of metals and pigments.

On the contrary, gelatin is an excellent growth substrate for several bacterial genera (*Bacillus*, *Clostridium*, *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Pseudomonas*, *Aeromonas*, *Serratia*, *Burkholderia*, *Yersinia* and *Salmonella*), which are named “gelatin liquefiers”. De Clerck and De Vos (2002) reported gelatin contamination by endospore-forming aerobic *Bacillus* spp. Such

long-term survivors can constitute a risk for photographic material. Abrusci and co-workers (2005) characterized the microbial populations of black and white motion picture films belonging to the Spanish cinematography Archives by combining morphological, biochemical and molecular-based methods. These authors found that all the isolated fungi (*Aspergillus*, *Penicillium*, *Trichoderma*, *Cladosporium*, *Mucor*, *Alternaria*, *Phoma* and *Cryptococcus*) were able to degrade gelatin, whereas only 7 bacterial strains (belonging to the *Bacillus* and *Staphylococcus* genera), out of a total of 14 isolated from the film, displayed gelatinase activity. Gelatinase efficiency was established by means of both viscosity decay profiles (Abrusci et al. 2004b and 2007) and chemiluminescence emission (Abrusci et al. 2007).

Borrego et al. (2010) studied the microbial population that colonized the inside of the Photographic Library of the National Archive in Cuba. Samples were collected in the air (by means of a sedimentation method) and on the surface of the photographs (using cotton swabs). All the microbial isolates were tested to establish their cellulolytic, proteolytic and amylolytic activities. They found a prevalence of proteolytic strains in the photographic material. Only one Gram-negative rod (namely *Pseudomonas* spp) was found on the considered samples. On the other hand, the air samples were colonized abundantly by cellulolytic fungi (which were also acid- and pigment-producers).

Bučková and co-workers (2014) used variable pressure scanning electron microscopy (SEM) analyses coupled with PCR DNA amplification and 16S rRNA (for bacteria), or ITS (for fungi), to characterize the microbial populations present in photographs housed in the “Archivio ente EUR” and “Archivio Centrale dello Stato” in Rome. A significant number of fungal genera, among which *Geotrichum*, *Aspergillus*, *Penicillium*, and the unusual *Zygosporium* were found, as well as bacteria (with a predominance of *Pseudomonas*) on documents that had previously been damaged by water. Any attempt to cultivate these strains was unsuccessful. Curiously, both *Geotrichum* and *Pseudomonas* were present in high abundance, thus suggesting that they were selected because of their resistance to silver ions.

### ***Synthetic polymer-based modern artworks and human history proofs: the risk of xenobiotic-degraders.***

Plastic objects, which are frequently present in contemporary art collections as important symbols of history, have recently revealed a risk of deterioration that is comparable with or even higher than that of ancient artworks. Apart from photo-degradation and oxidation, biological deterioration also accounts for damage. Pigments and microbial biofilms are often responsible for superficial damage, but the main problem arises when plastic material is used by microorganisms as

a nutrient for growth. The recent environmental emergency situation has prompted the search for biodegradable plastic polymers, together with efforts to select bacteria that are able to hydrolyze recalcitrant xenobiotic molecules (Yoshida et al. 2016). These bacteria, which generally release acids from their oxidative catabolism, can thus also cause the degradation of high-value plastic items (Cappitelli and Sorlini 2008). As mentioned in the section in which textiles are discussed, polyurethane (Rowe and Howard 2002), polyvinylchloride (PVC) (Webb et al. 2000), nylon (Friedrich et al. 2007) and even PET (Yoshida et al. 2016) can undergo bacterial or fungal colonization and degradation by means of peculiar enzymatic activities, such as urease, esterase and manganese peroxidase (Cappitelli and Sorlini 2008). Spacesuits (Fig. 5), compact discs, Barbie dolls and other toys can be colonized by fungi and bacteria (e.g. *Bacillus subtilis* and *Pseudomonas aeruginosa*) that irreversibly destroy the objects (Breuker et al. 2003; Garcia-Guinea et al. 2001; McCain and Mirocha 1994; Webb et al. 2000). Both *Cladosporium* and *Paecilomyces spp* were identified, by means of traditional methods, on astronauts' suits (Breuker et al. 2003), whereas fluorescent *in situ* hybridization was necessary to identify cyanobacteria and archaea in more complex matrices (Cappitelli et al. 2006). However, since microbial colonization is not always associated with a clear biodeterioration, precious information can be obtained by evaluating the material damage using electronic microscopy, viscosity assessment, differential scanning colorimetry and infrared spectroscopy (Cappitelli and Sorlini 2008). All these data suggest that modern specimens, which constitute a feature of a historical period (1950-today), require adequate strategies to contain their deterioration.

## CONCLUSIONS

What do an astronaut's spacesuit, a Viking ship, a shroud, a compact disc, a medieval crypt and a cinematographic film have in common? Regardless of their natural or synthetic origin, they all undergo different forms of deterioration, including microbial degradation. This review article has reported the main biochemical activities involved in cultural heritage biodeterioration, highlighting the importance of cellulases, collagenases, gelatinases, esterases and other enzymes as well as the metabolic pathways of microorganisms in this process. In a period in which the attention of researchers is focused on the synthesis of biodegradable polymers, as well as on the selection of xenobiotic degraders, this mini-review underlines the fragility of modern synthetic man-made objects, which risk having a shorter life than 5000 year-old stone monuments. Progress in this research field is an essential requisite for the preservation and restoration of artistic and cultural heritage items for future generations.

## COMPLIANCE WITH ETHICAL STANDARDS

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631

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840 **Figure Legend**

841 Fig. 1. Oseberg Viking ship exposed in the Viking Ship Museum at Bygdøy in Oslo (Norway).

842 Fig. 2. Schematic representation of three commonly used molecular-based procedures for bacteria  
843 identification in cultural heritage samples. a) Denaturing Gel Gradient Electrophoresis (DGGE); b)  
844 Restriction Fragment Length Polymorphism (RFLP); c) Fatty Acid Methyl Esther Analysis (FAME).

845 Fig. 3. Bacterioruberin pigments (a) produced by bacterial cultures of *Rubrobacter radiotolerans* (b)  
846 and which contaminate the 9<sup>th</sup> century frescoes of the Crypt of the Original Sin Chapel near Matera  
847 (Italy) (c).

848 Fig. 4. Black and white and color photographic films at risk to microbial deterioration.

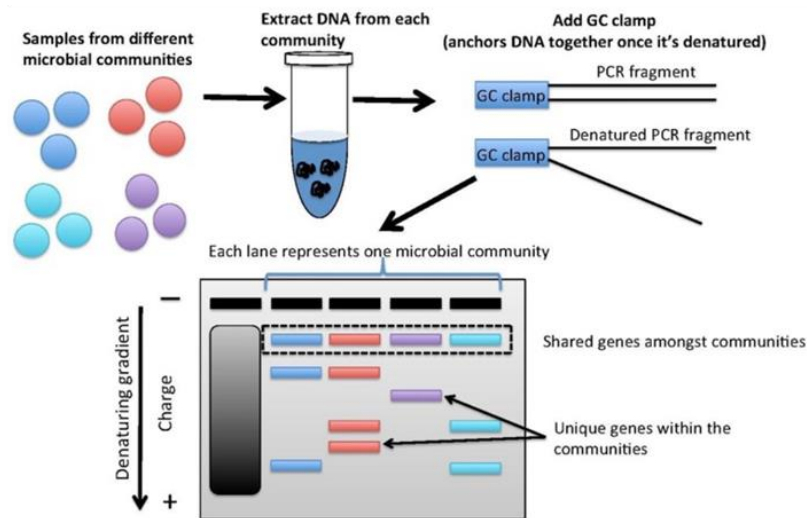
849 Fig. 5. Apollo spacesuit on which fungal contamination has been ascertained.



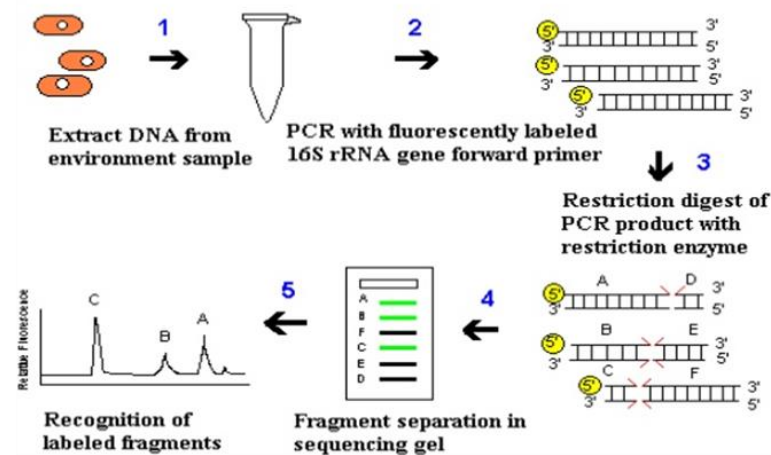
850 Fig. 1



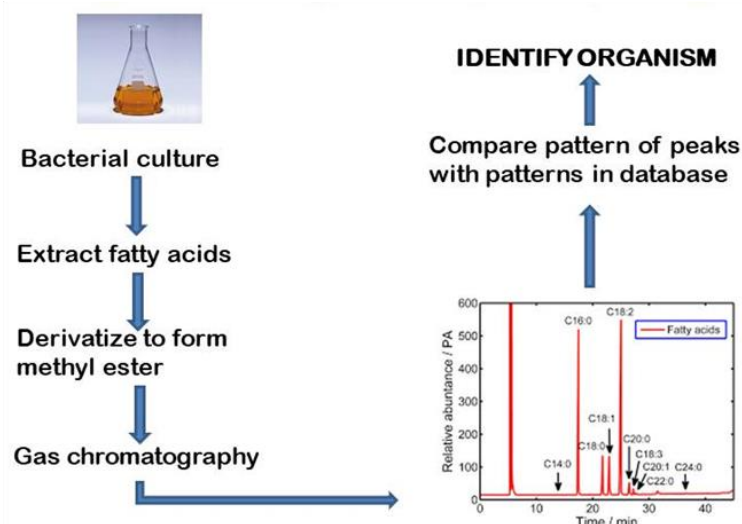
## a Denaturing Gel Gradient Electrophoresis (DGGE)



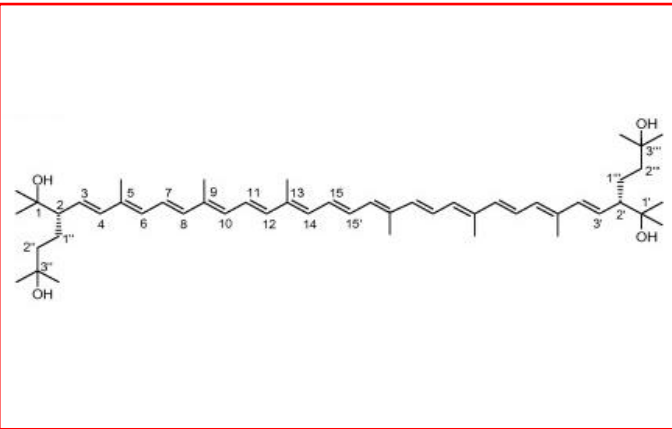
## b Restriction Fragment Length Polymorphism (RFLP)



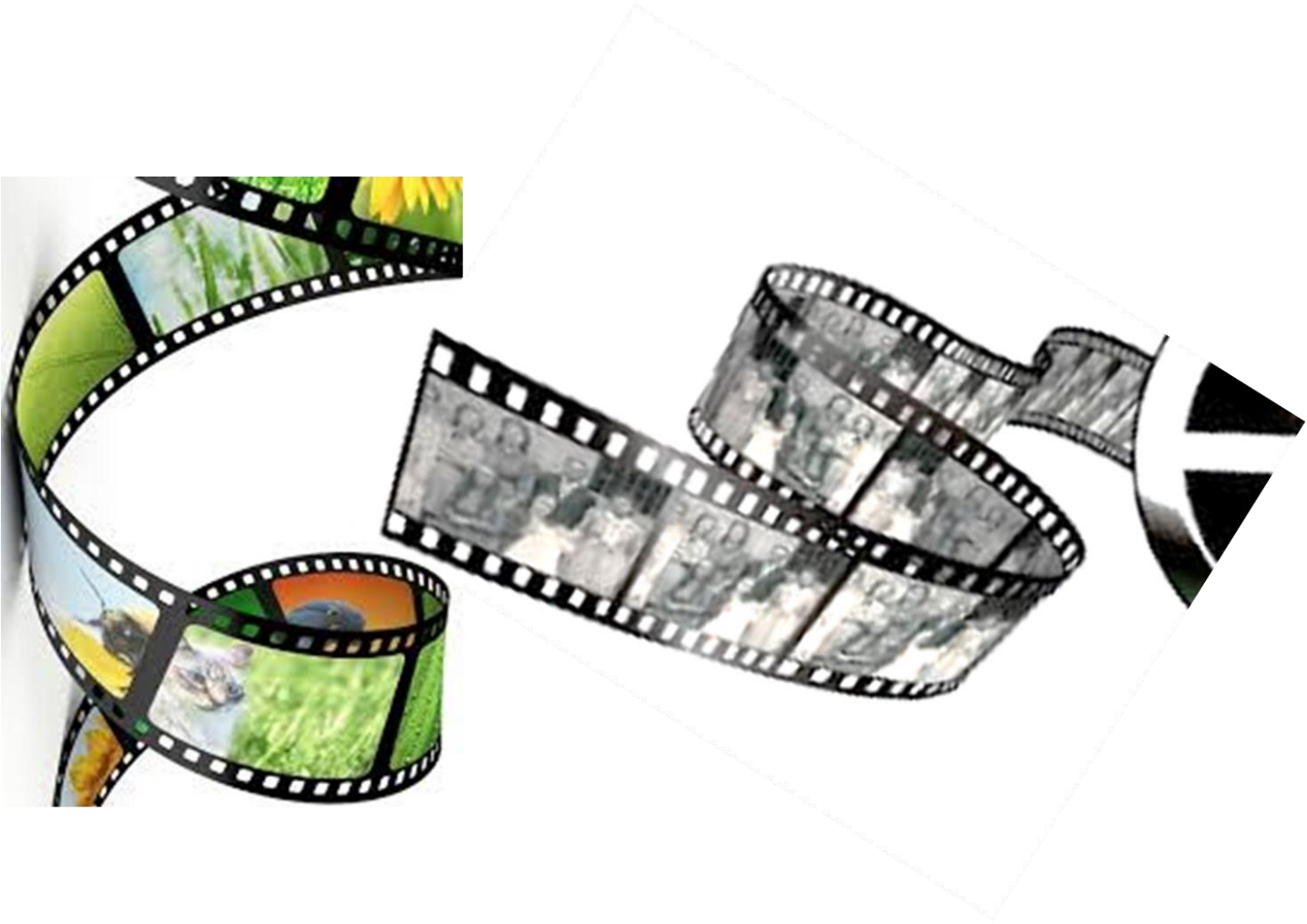
## c Fatty Acid Methyl Ester (FAME) Analysis



852 Fig. 3



863 Fig. 4





865 Fig. 5

