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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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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

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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.

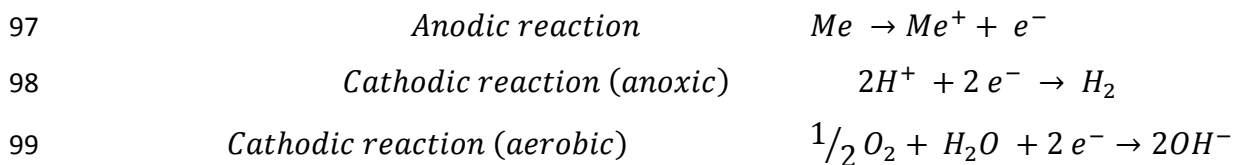
Autotrophic organisms may be the starters of syntrophic chains. Chemoautotrophs (such as the acid-producing sulfur-oxidizing and nitrifying bacteria that dissolve the alkaline material of stone) can be involved, but photoautotrophs, such as cyanobacteria, are better adapted to the oligotrophic and dry 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

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

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

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

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



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

119 Microbial-induced corrosion mainly concerns buried, sunk or poorly conserved metallic
 120 antiquities or artworks (Del Junco et al. 1992). Uncommon corrosion products, such as Mackinawite
 121 (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 15th and 11th 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^{2+} 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_4 . 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-SO₄ 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.

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

157 Lignocellulosic material is the main component of paper, vegetal textiles and wood.
158 Lignocellulose mainly includes cellulose, hemicellulose and lignin, whose relative amounts may
159 widely vary depending on the specific item (Bomble et al. 2017). It is an energy/carbon substrate for
160 many different microorganisms, including both bacteria and fungi. These organisms may damage
161 library book collections, ancient documents, drawings and photographs (Cappitelli et al. 2010), as
162 well as wooden objects, e.g. ancient coffins, weapons, Native American houses, boats, bridges, ships
163 and shipwrecks (Björdal 2012a and 2012b; Björdal et al. 1999; Palla et al. 2013; Singh 2012).
164 Lignocellulose deconstruction in the biosphere is a complex phenomenon which is generally
165 catalyzed by mixed microbial communities, in which each strain provides its peculiar enzyme
166 activity(ies) (e.g. lignin and/or cellulose and/or hemicellulose depolymerizing action) (Bomble et al.
167 2017). The best characterized lignin degraders are white-rot and brown-rot fungi which use oxidative
168 mechanisms, i.e. peroxidases and laccases or Fenton chemistry, respectively (Bomble et al. 2017).
169 Hemi-/cellulolytic microorganisms mainly biosynthesize glycoside hydrolases and polysaccharide
170 lyases, although other biochemical mechanisms for hemi/cellulose depolymerization have been
171 recently discovered (Bomble et al. 2017). Cellulolytic organisms are also involved in the deterioration
172 of fabric of vegetal origin. However, this aspect will be treated in more detail in the next section
173 (textile deterioration).

174 Wood

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

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

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

241 Paper

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

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

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

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

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

283 As far as pigmentation is concerned, tents, sails and beach umbrellas, being exposed to sun-
284 light and humidity, can support the growth of algae that generate green pigments, whereas raw wool
285 (fleece) can be colonized by *Pseudomonas aeruginosa*, which generates both green (in an alkaline
286 environment) and red (in acidic conditions) pigments during wool degradation. Yellow, orange,
287 brown or black pigments can also be synthesized by *Brevibacterium*, *Bacillus*, *Rhodococcus*,
288 *Corynebacterium*, *Achromobacter*, *Streptomyces*, and by fungi such as *Rhodotorula*, *Penicillium*,
289 *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

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

329 *Wool and silk*

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

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

341 As far as silk is concerned, its main protein fibroin is made up of fibers held together by
 342 sericin, a second protein that acts as an adhesive. While fibroin is essentially constituted (more than
 343 90%) by four amino acid repeats (glycine, alanine, serine and tyrosine) that are less attractive as
 344 microbial food, sericin is the first one to be utilized as a nutrient by microorganisms. Degummed (i.e.
 345 sericin-deprived) silk is degraded at a slower rate, and two months are required before a decrease in
 346 strength can be detected. Nevertheless, sericin-deprived silk is more susceptible to light damage.
 347 Although only *Pseudomonas cepacia* can use fibroin as a carbon source, *Bacillus*, *Serratia*,
 348 *Pseudomonas* and *Streptomyces* have also been found in a degradation mixture, thus suggesting the
 349 occurrence of co-metabolization (Forlani et al. 2000; Seves et al. 1998). One fungal strain of
 350 *Aspergillus niger* has also been described as being able to modify the fibroin structure (Szostak-
 351 Kotowa 2004).

352 *Man-made textiles*

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

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

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

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

390 ***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 μm 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

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

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

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

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

452 Several alternative approaches have been employed/developed to characterize the causative
453 agents of biodeterioration. In 1996, Rölleke and co-workers characterized the microbial population
454 on a 13th century wall painting belonging to the Chapel of the Herberstein Castle in Austria. By means
455 of electron microscopy, they detected bacteria that had a filamentous morphology. Culturing allowed
456 the growth of only five strains, three of which gave rise to pigmented colonies (white, yellow and red,
457 respectively). DGGE analysis on the amplified DNA from the purified isolates revealed the presence
458 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 17th 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 9th 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-CH₃ 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*), α -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.***

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

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

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

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

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

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

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

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

585

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

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

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

613 **CONCLUSIONS**

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

625

626 **COMPLIANCE WITH ETHICAL STANDARDS**

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631

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- 839

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 9th century frescoes of the Crypt of the Original Sin Chapel near Matera
847 (Italy) (c).

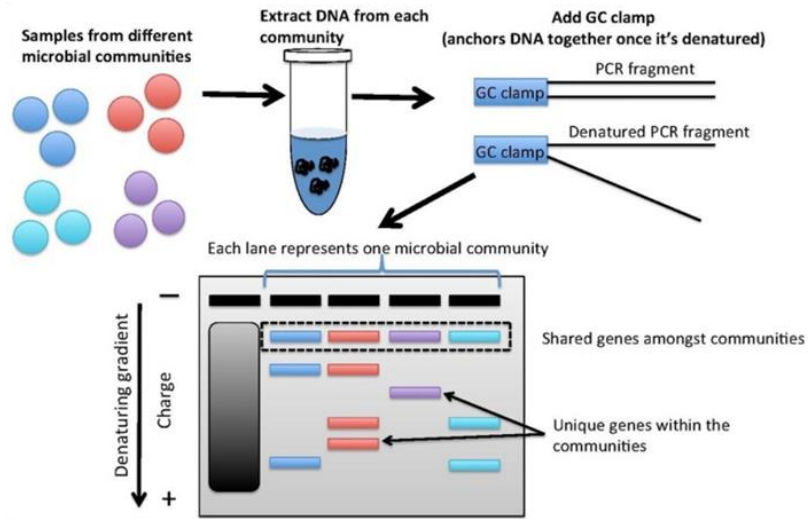
848 Fig. 4. Black and white and color photographic films at risk to microbial deterioration.

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

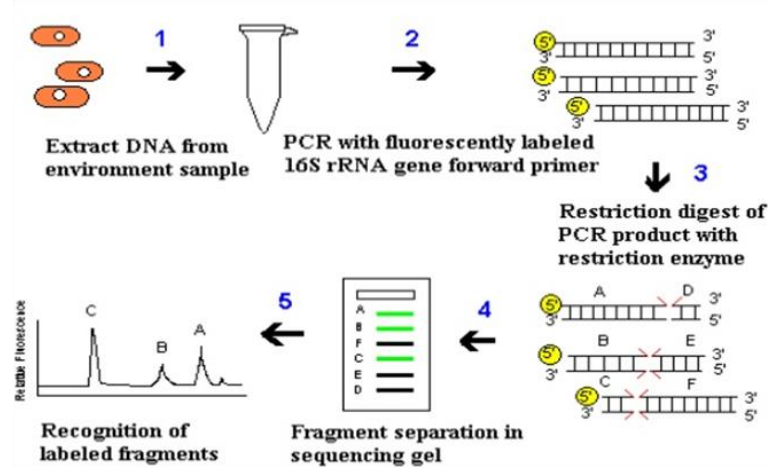


850 Fig. 1

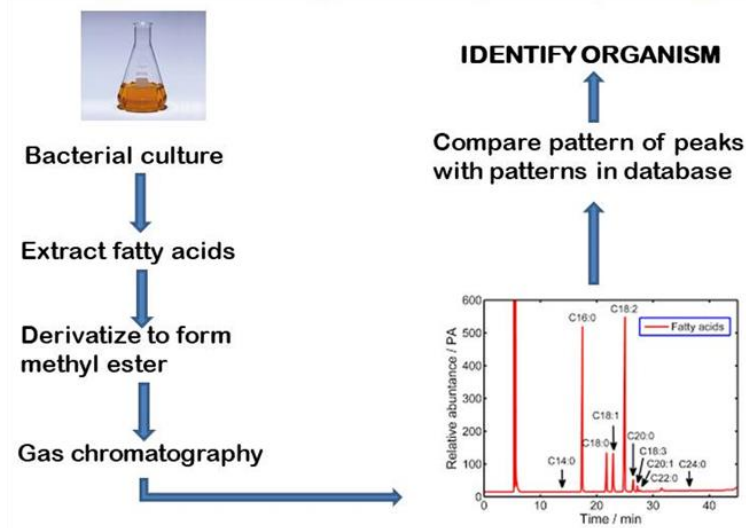
a Denaturing Gel Gradient Electrophoresis (DGGE)



b Restriction Fragment Length Polymorphism (RFLP)



c Fatty Acid Methyl Ester (FAME) Analysis



851 Fig. 2

852 Fig. 3

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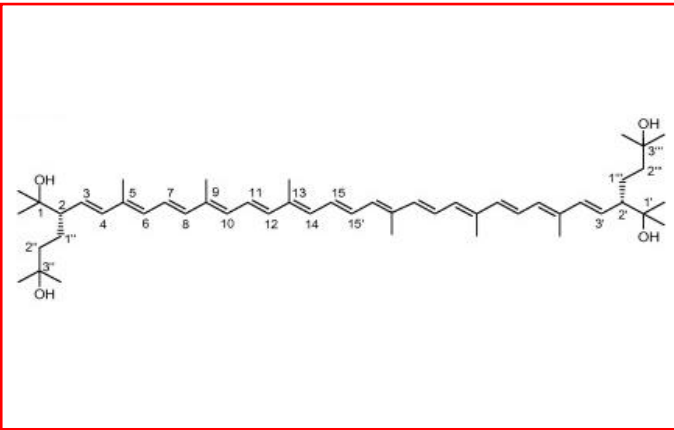
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863 Fig. 4



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865 Fig. 5

