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

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:

$$Anodic\ reaction \qquad Me \rightarrow Me^+ + e^-$$

$$Cathodic\ reaction\ (anoxic) \qquad 2H^+ + 2\,e^- \rightarrow H_2$$

$$Cathodic\ reaction\ (aerobic) \qquad {}^1/_2\,O_2 + H_2O + 2\,e^- \rightarrow 2OH^-$$

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

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

The study by Marvasi et al. (2009) has shown that the bacterial colonization of medieval stained glass windows in Florence cathedral was favored by dust, crusts and organic matter. The inside of the windows did not exhibit any visible damage, whereas the outside of the glass was clearly contaminated with crusts, except for the green parts of the windows where no damage was detected, even on the external parts. Hence, the authors analyzed the chemical composition of the green glass, hypothesizing an antibacterial activity of the glass component(s). Copper (present in high quantities in green glass), which in its Cu^{2+} form is toxic for microbial cells, and has been demonstrated to reduce colonizer biodiversity (Milanesi et al. 2006), was not the cause of the lower number of microorganisms that were found, since most of the bacteria were resistant to $CuSO_4$. Similarly, lead (Pb) was not involved, since it was only present on the internal side of the glass. However, a higher Na content was found in the green glass than in the other colored glass. The Na-rich glass also displayed a higher silica content (around 65%) than the K-rich glass. In particular, the green glass was found to be of the so-called Na-rich alkaline silicate-sodium type of glass that is typical of the medieval Renaissance period. This condition is unfavorable for microbial growth. Conversely, the K-Ca-SO_4 crusts found on the other colored glass (ascribable to gypsum and syngenite, as determined by means of Fourier Transform Infrared Spectroscopy, FTIR) created a microenvironment that was able to retain nutrients, microorganism and moisture, which in turn protected the bacteria from the high temperatures reached as a result of exposure to sunlight during the day. The combination of microscopic/biochemical identification with 16S rDNA-based molecular tools indicated that the populations found inside the cathedral were different from those found on the outside glass; *Firmicutes* in particular were absent on the inside windows. The clean glass (inside and green) mainly hosted *Actinobacteria* and *Proteobacteria*. The former were previously also found on other Cathedral windows (Krumbein et al. 1991; Rölleke et al. 1999), whereas the proteobacteria *Brevundimonas* are typical of alkaline and nutrient-poor environments (Abraham et al. 1999). A high number of spore-forming *Bacillus* and *Paenibacillus* were found in the crusts, thus indicating that the sporulation 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.)

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

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

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

In 2004, Helms and coworkers analyzed anaerobic bacteria that colonized an ancient wooden 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 depolimerize 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 sun-light 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

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

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

Cotton, linen, jute and hemp

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

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

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

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

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

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

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

An interesting paper by Imperi et al. (2007) reported the characterization of both bacteria and pigments detected on a 9th century fresco, illustrating scenes from the Genesis (Fig. 3). These byzantine paintings, discovered in 1963 in the Crypt of the Original Sin near Matera (Italy), had suffered from water infiltration, carbonate precipitation and discoloration. Former attempts to characterize the microflora, by means of morphological and culture-based methods, had revealed the presence of cyanobacteria and green algae. Later, an unwanted reddish pigmentation that covered much of the painted area appeared. Background-subtracted *in situ* micro Raman spectra of the pigmented area revealed three major bands, ascribable to the vibrational mode of the C-CH_3 groups, to the single C-C bonds and the double C=C bonds, respectively. The analytical results made it possible to conclude that the pigments were carotenoid molecules. Both ARDRA and DGGE were used for microbial typing. Actinobacteria (in particular *Rubrobacter radiotolerans*), α-Proteobacteria (in particular *Erythrobacter* spp), Bacteroidetes (in particular *Sphingobacterium*) and Cyanobacteria were found to be present, as well as Archea such as *Halococcus* and *Haloferax*. However, Archea only represented a numerically insignificant contaminant (less than 0.1% of the 16S rRNA gene pool), whereas *Rubrobacter radiotolerans* was abundant (about 87% of the 16S rRNA gene pool per sampled site) in almost all the samples from the pigmented area. In order to better assess the cause of the pigmentation, pigments produced by *Rubrobacter radiotolerans* were analyzed by micro Raman spectroscopy, and it was demonstrated that they were the same as the pigmented area on the fresco. These carotenoids, named bacterioruberins, have a C-50 length and display 13 conjugated double bonds. However, this result cannot exclude that other microbial strains (eubacteria, such as *Micrococcus* and *Arthrobacter* and archea like *Halococcus* and *Haloferax*) could also synthesize ruberins, since the Raman analysis was unable to distinguish bacterioruberins from different species.

Motion picture films and photographic material biodeterioration: the contribution of 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 19th 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

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 chemioluminescence emission
567 (Abrusci et al. 2007).

568 Borrego et al. (2010) studied the microbial population that colonized the inside of
569 thePhotographic 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 scansion 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

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 restauration of artistic and cultural heritage items for future generations.

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).

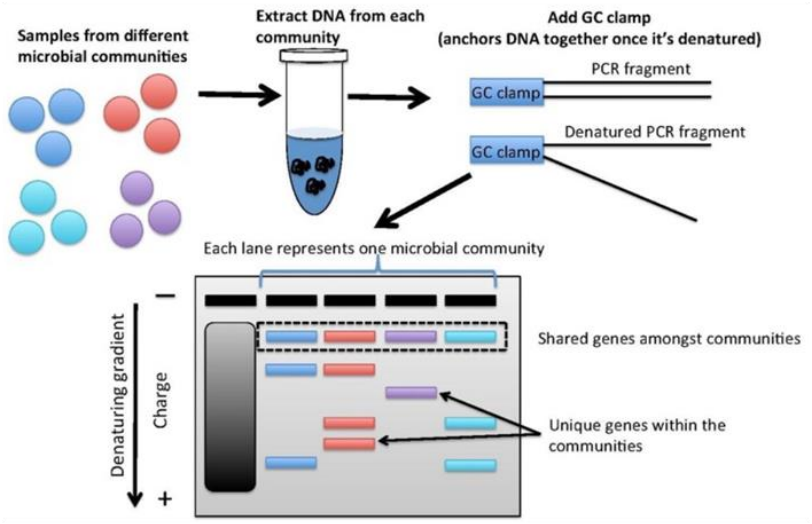
845 Fig. 3. Bacterioruberin pigments (a) produced by bacterial cultures of *Rubrobacter radiotolerans* (b)
846 and which contaminate the 9th century frescoes of the Crypt of the Original Sin Chapel near Matera
847 (Italy) (c).

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

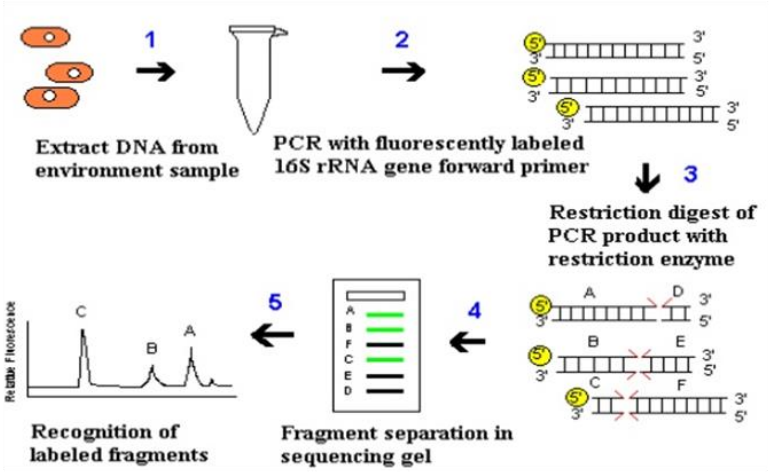
849 Fig. 5. Apollo spacesuit on which fungal contamination has been ascertained.

850 Fig. 1

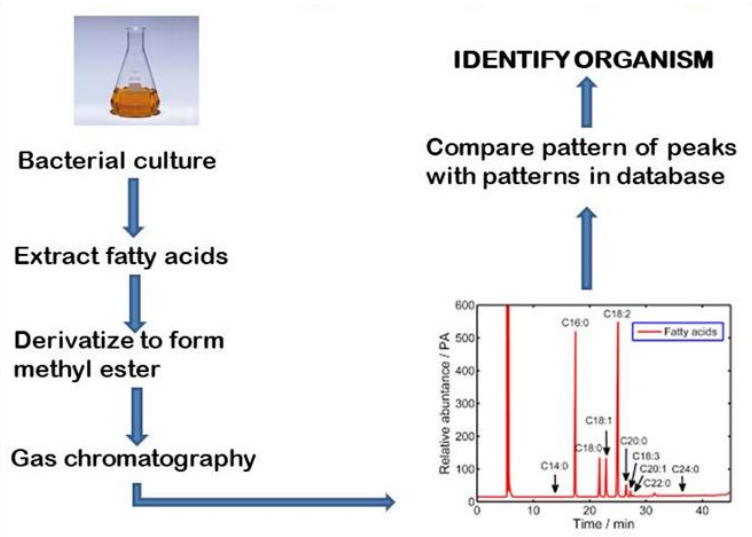
a **Denaturing Gel Gradient Electrophoresis (DGGE)**



b **Restriction Fragment Length Polymorphism (RFLP)**

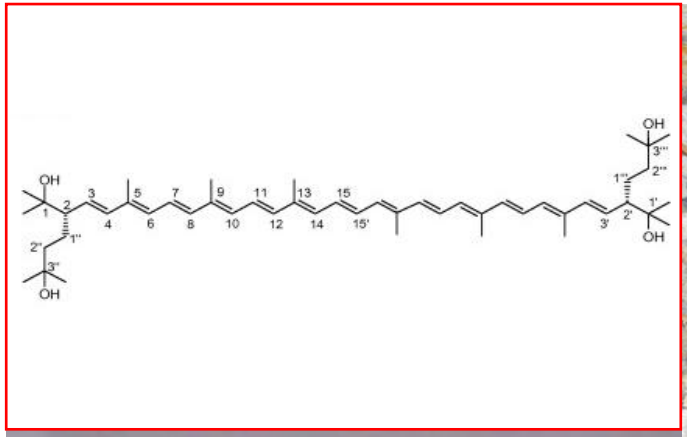


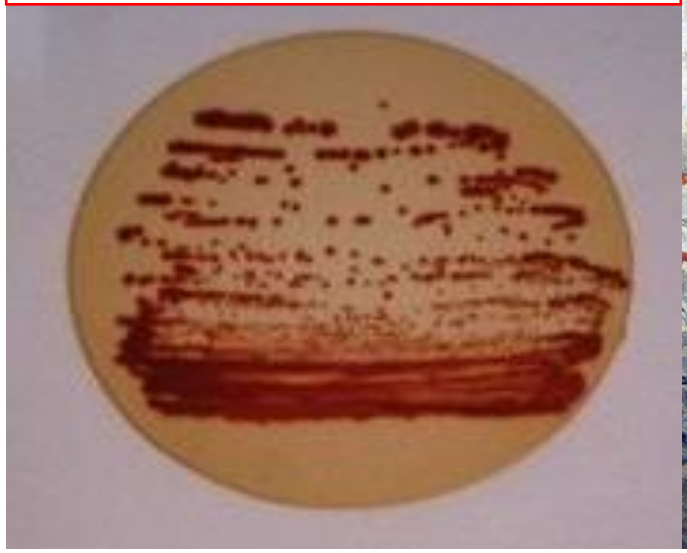
c **Fatty Acid Methyl Esther (FAME) Analysis**



851 Fig. 2

Fig. 3





863 Fig. 4

864

865 Fig. 5

866