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Biodeterioration of modern materials in contemporary collections: can biotechnology help?

Francesca Cappitelli, Pamela Principi and Claudia Sorlini

Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche, Università degli Studi di Milano, Via Celoria 2, 20133 Milan, Italy

Contemporary collections frequently contain man-made materials. Although synthetic materials are considered more resistant to chemical, physical and biological damage than natural materials, they can also undergo rapid deterioration. In this Opinion article, we claim that biotechnology can help to identify biodeteriogens and prevent colonisation of polymeric surfaces through the application of biological products that reduce cell adhesion. We report the study of 'Futuro', made in 1965 by the Finnish architect Matti Suuronne. This ski-cabin, constructed of glassfibre-reinforced polyester, polyester-polyurethane, and poly(methylmethacrylate), was significantly degraded by conspicuous growth of microorganisms, identified as Cyanobacteria and Archaea using fluorescent *in situ* hybridisation. Ultimately, if biodeteriogens are able to adhere to the polymer surfaces, molecules with enzymatic activity can help to prevent the formation of biofilms – a main cause of deterioration – and aid the work of the conservator.

Introduction

Contemporary art crosses the boundaries of medium; it is not limited by materials or methodology. It might or might not use traditional forms and can engage performance, installation, video or all other materials or media imaginable. Modern materials, spanning from cellulose derivatives to synthetic polymers, have become increasingly important in collections. Thus, contemporary objects containing a wide variety of materials present countless challenges in handling, preservation, conservation, storage and exhibition.

Despite what is generally thought, curators have begun to notice that objects made of plastics degrade with time, sometimes rapidly, and have generally a useful lifetime of between ~1 and 40 years [1].

Microorganisms are among the various agents capable of degrading these materials – such biodeteriorations might act in synergy with physical and chemical means of deterioration to cause increased damage. Recent investigations led to the identification of plastic-degrading microflora and the discovery of genes encoding enzymes involved in the degradation of synthetic polymers [2].

The conditions in which artistic objects are currently stored have proved to be the most suitable for traditional cultural heritage. However, regarding contemporary objects (magnetic and optical discs, toys, design furniture etc.), there are no widely accepted standard storage conditions because these conditions have only been considered a concern in the past few years [3,4].

In the following, we illustrate two cases of the biodeterioration of contemporary art objects. First, we report the case of 'Propagazioni', created in 1997 by Giuseppe Penone and owned by the Galleria Civica d'Arte Moderna e Contemporanea in Torino, Italy (www.gamtorino.it/). Some areas of this artwork were intentionally exposed to flowing water by the artist, and because of an unexpected event at a temporary place of exhibit, the wet surfaces presented with massive microbial growth and dramatic discoloration. The problem was impossible to solve, and the museum proposed that the artist replaced the damaged parts of the work, which is now back in exhibit (Antonio Rava, personal communication).

The second case occurred at the National Air and Space Museum of the United States Smithsonian Institution, which preserves the largest collection of spacesuits in the world (http://www.nasm.si.edu/). Historically significant cloths from the Apollo lunar missions suffered from colonisation by *Paecilomyces* and *Cladosporium* – growth of these on the synthetic fibres was observed using a scanning electron microscope [5].

The microbial contamination of modern materials in contemporary collections is still an underestimated concern. One reason for this is the difficulty in establishing a definitive causative relationship between the biological agents and the damage to materials, particularly in those cases where there is damage but no more biological growth.

In addition, to consolidate and protect traditional artistic objects, conservators have sometimes made indiscriminate use of synthetic materials. The result is that the current conditions of treated objects are now sometimes even worse than the conditions of non-treated objects and, often, biodeterioration of the added materials is a cause of the damage [6,7]. Thus, it is clear that preventive conservation of modern materials should include methods to reduce the development of microbial colonisation.

Is this a biological problem?

If biological damage is suspected, the following questions should be asked: is this really a biological problem? how

Corresponding author: Cappitelli, F. (francesca.cappitelli@unimi.it).
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can we get rid of the bugs? how can we prevent future biological damage? We hope to shed some light on these important issues.

In recent years, in addition to the common approach of using visual inspection and measuring physical effects to evaluate the biodeterioration of synthetic polymers other techniques have been used, including vibrational spectroscopies – particularly photoacoustic spectroscopy [8]. During the past decade, molecular biology techniques, which often provide more complete and reliable information than traditional methods, have been an important diagnostic tool available to conservators for the study of the biodeterioration of traditional cultural heritage [9,10]. To the best of our knowledge, biomolecular techniques have, so far, never been applied to the study of the biodeterioration of modern materials in contemporary art objects. Knowledge of the microbial susceptibility of modern materials is the bare minimum required to plan proper preservation and conservation treatments [8]. Among the various techniques for studying microbial ecology, we think that fluorescent *in-situ* hybridisation (FISH) is one of the most promising [11]. In this technique, the fluorochrome-labelled probes – the sequences of which are complementary to specific phylogenetic groups, from the domain to the genus level – bind to the target RNA, enabling the detection and the identification of the whole cell using epifluorescence microscopy. The use of specific probes for the different domains (Archaea, Bacteria and Eukarya) gives precious information on the structure of the microflora involved in the biodeterioration process. In addition, this technique is rapid and, with suitable sampling devices (e.g. adhesive tape strips), can provide the spatial distribution of the microbial community without causing damage to the objects [12]. Below, we present the study of the biodeterioration of 'Futuro' using FISH.

Futuro

The plastic ski-cabin Futuro was designed by the Finnish architect Matti Suuronnen in 1965 (Figure 1). The exterior consists of glassfibre-reinforced polyester filled with polyester-polyurethane foam and includes windows of poly(-methylmethacrylate) (Perspex). The interior also contains

Figure 1. Futuro by the Finnish architect Matti Suuronnen, 1965.

Figure 2. Detail of Futuro showing dramatic evidence of microbial growth.

various plastics. Futuro represents an extraordinary document of its time, of which fewer than 15 examples remain. The Museum Die Neue Sammlung in Munich (http://www.die-neue-sammlung.de/z/enindex.htm) is currently conserving 'number 13', which shows significant damage caused by microbial growth (Figure 2), largely due to exposure – the artwork is still located outdoors. Extensive investigations of the degradation processes were carried out by The Museum Die Neue Sammlung, with The Netherlands Institute for Cultural Heritage (http://www.icn.nl) performing chemical analysis, and our Department (http://www.distam.unimi.it) conducting biotechnological investigations.

The suspected biological nature of the alteration was confirmed by optical microscope observations and 4′,6-diamidino-2-phenylindole (DAPI) staining using a digital epifluorescence microscope (Leica DM4000B), equipped with the CoolSnap CF camera (Photometrics, Roper Scientific), pictures were acquired with the software RS Image Ver. 1.7.3 (Roper Scientific, Inc.). Phylogenetic identification at the domain and division levels was obtained by applying *in situ* hybridisation with several probes: we used a specific probe for Archaea domain, ARCH915; a probe for Bacteria domain, EUB338; and a set of probes specific for the Cyanobacteria phylum, CIV/V, CYA361, CYA664, and CYA 762 (probe details are accessible at http://www.probebase.net) [13]. DAPI staining used in conjunction with probes EUB338 and ARCH915 showed that the eukaryotic component of the microbial community was insignificant. Aggregate-forming coccoid cells in the range of 2–5 um in diameter, and 5 um diameter cells in filaments, were identified as Cyanobacteria (Figure 3), and smaller cells (between 0.5 and 1 um diameter), grouped in clusters, were identified as Archaea (Figure 4). In all the samples, Cyanobacteria cells comprised >90% of the cells identified as belonging to the Bacterial domain, identified by means of hybridization with the domain specific probe EUB338. In a few samples, Archaea seemed to be the densest taxa but the quantification of the Archaea cells could not be achieved to statistical significance due to their small dimensions and grouping into clusters.

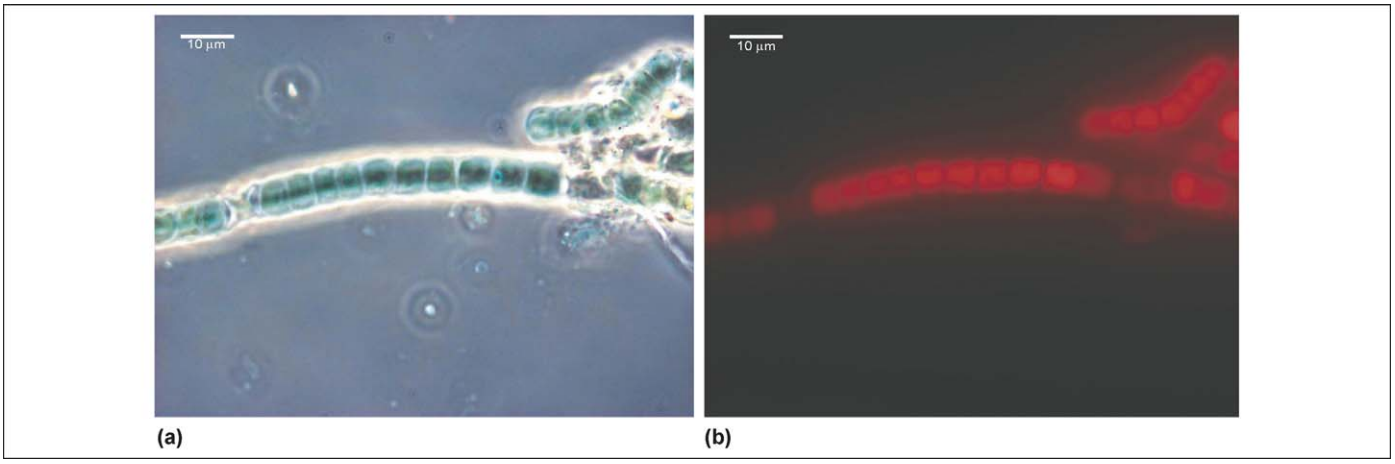

Figure 3. Cells in a filamentous growth visualized with **(a)** phase contrast (1000×) and **(b)** epifluorescence (1000×). Cells positive for hybridisation with a Texas Red fluorochrome-labelled probe specific for Cyanobacteria appear red on the dark background. Probe labels: CIV/V, CYA361, CYA664, and CYA 762 (http://www.probebase.net). Bar = 10 μm.

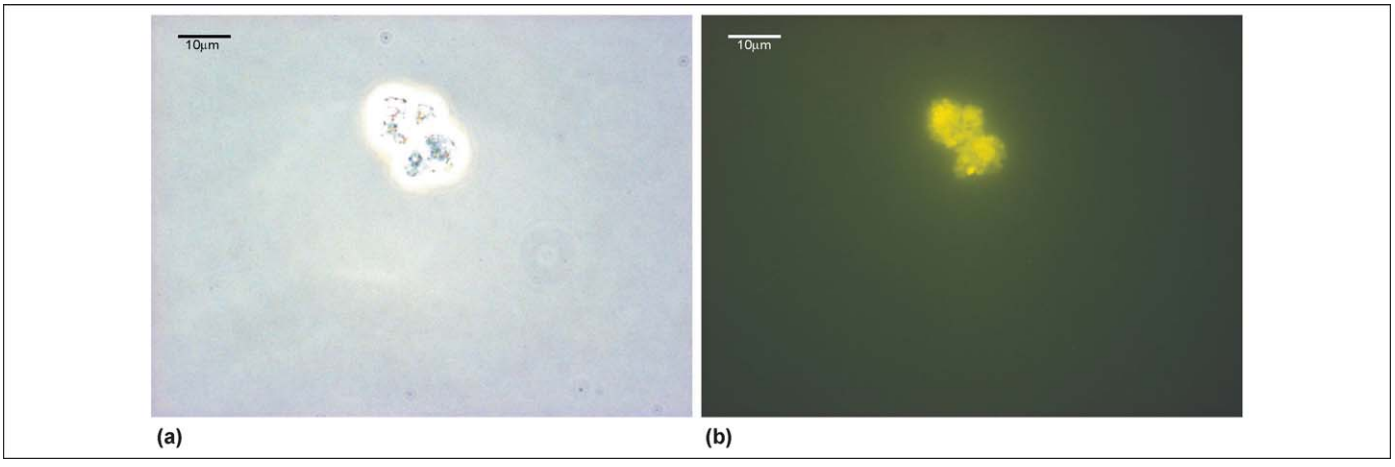

Figure 4. Microbial cluster visualized in **(a)** phase contrast (1000×) and **(b)** epifluorescence (1000×). Cells positive for hybridisation with fluorescein-labelled Archaea-domain-specific probe appear green on the dark background. Probe label: ARCH915 (http://www.probebase.net). Bar = 10 μm.

Cyanobacteria produce extracellular polymeric substances (EPSs) that are crucial in different processes, including surface colonisation, cell aggregation and biofilm formation and stabilization. Biofilms are encased microcolonies of microbial cells attached to an inert or living surface by way of adhesive polysaccharides excreted by the cells. EPS cause chemical damage because the negatively charged polysaccarides are able to chelate cations and, being organic matter, support further microbial growth. For these reasons, Cyanobacteria are well-known biodeteriogens – organisms that cause a detrimental change to a work of art because of their physical and/or chemical activity – of cultural heritage [14,15].

Archaea have developed a variety of molecular strategies to survive in the harsh environments in which they are found naturally. In addition to simple substrates, certain polymeric substances can be degraded by Archaea, and some species produce extracellular enzymes such as proteases, esterases, glycosyl hydrolases, cellulose-degrading enzymes, xylan-degrading enzymes and chitinases [16]. Thus, owing to the variety of enzymes produced, some Archaea might also attack synthetic polymeric materials.

Conservators at The Museum Die Neue Sammlung are wondering about the future location of Futuro. It is evident that, after the biological agents have been removed, if the ski-cabin is kept outdoors without changing any of the environmental conditions the same biological problem will occur again in a few months time. However, if the piece was moved to an inside location, the current context would be lost.

How can we get rid of the bugs?

Microbial cells can often be dislodged from surfaces either mechanically or using surfactants; furthermore, the biofilm structure can be dampened using enzymes or chelating agents of divalent cations. However, for decades, microbial abatement has been commonly achieved by the application of biocides – chemical substances used to kill living organisms. The concern about the use of biocides is that, eventually, they are released into the environment and because they are generally not specifically targeted against biodeteriorating microorganisms, they are potentially dangerous for human health and the environment. In addition, it is known that biofilms are more resistant to

antimicrobials than planktonic cells [17]. Many factors are involved in biofilm resistance, including the exopolysaccharide matrix, which delays the penetration of the biocide, and the slow growth of the cells in the biofilm.

Previously, the use of biocides to control biodeterioration has been restricted by European regulations: Council Directive 91/414/EEC (published in 1991) states that active substances cannot be released into the environment unless they are included in a positive EU list. An EU-wide programme of evaluation to create this list is underway. Consequently, since 1991 several pesticides that have been used in past conservation treatments have been withdrawn from the market; this awareness of pesticide hazards has led to the search for alternatives.

Cyanobacteria are the main target biodeteriogens in the conservation of Futuro; therefore, compounds that specifically target photosynthetic microorganisms should be considered. Oscillatorin, a secondary metabolite isolated from the filamentous cyanobacterium *Oscillatoria laetevirens*, inhibits photosystem II activity of Cyanobacteria and green algae and, therefore, is a possible agent [18].

How can we prevent future biological damage?

Previous approaches have concentrated on trying to kill microorganisms but the trend now is to either disarm them or plan preventive strategies. Because bacterial adhesion is a prerequisite for biofilm formation, the prevention of microbial adhesion on surfaces has a major impact in preventing damage. Another interesting alternative to chemical biocides are compounds produced by living organisms. Marine eukaryotes, such as sponges, seaweeds and molluscs, are constantly faced with potential fouling microorganisms and, in response, they have evolved strategies to defend themselves from being colonised. Natural marine products, such as halogenated furanones from the Australian red alga *Delisea pulchra* and zosteric acid or *p*-(sulphooxy) cinnamic acid from the eelgrass *Zostera marina* [19], can be exploited and used to reduce, dramatically, the attachment of microorganisms on polymeric surfaces [20]. Finally, tetraether lipids situated in the cytoplasmic membrane of the archaeon *Thermoplasma acidophilum* were suggested to be a novel approach for the prevention of biofilm formation by modification of surfaces. Modified silicone surfaces with covalently bound tetraether lipids actually reduced microbiological adhesion (Briese, B.H. (2004) Article presented at the International Conference Alternative and Conventional Anti-Fouling Strategies organized by the International Biodeterioration and Biodegradation Society in conjunction with the IWW Water Centre, Mülheim, Germany, 13–15 September 2004). Furthermore, sterile polymeric surfaces have been obtained that anchor long-chained hydrophobic polycation-containing, non-leaching antimicrobial monomers to the surface of the material [21]. This method presents clear advantages and environmental benefits compared with other methods of applying biocides. Biodeterioration is considered a real risk only if the extent of biofilm accumulation exceeds a certain threshold. An alternative prevention strategy is, therefore, to keep biofilm development below this level. One way to prevent microbial film development is the repression or inactivation of the cell-to-cell communication molecules (quorum sensing) involved in the formation of the biofilm [22]. When the cell density reaches a certain threshold, cells communicate among themselves by means of small signal molecules called autoinducers, for example, lactones. The disruption of bacterial quorum sensing comprises signal molecule degradation by specific enzymes such as bacterial lactonases. The future control of quorum sensing might also be useful in respect to the improvement of biocide effects [17]. To control biofilm formation and biodeterioration, monitoring non-destructive systems that provide online and real-time information would be a great advantage. This important improvement can be achieved using *in-situ* PCR [23] or fluorescence *in-situ* hybridisation [24].

Concluding remarks

In conclusion, the conservation of modern materials in contemporary objects – a unique and invaluable legacy, both of art and technology – deserves more attention. Biotechnologies can be of service not only in the diagnostic of microbial deteriogens but also in the prevention of microbial colonisation and, as a consequence, damage to the objects.

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