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A methodology to select bacteria able to remove synthetic polymers

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ABSTRACT

Synthetic polymers have often been used for the protection and consolidation of cultural heritage. Although it was generally thought that synthetic polymers were not susceptible to deterioration, there are now many papers in the scientific literature demonstrating the opposite. The degradation of synthetic polymers can be due to chemical, physical and biological factors. At present, the traditional way for removing a degraded synthetic polymer is the use of mixtures of solvents that pose some health risks. This work proposes a method to select bacteria able to remove synthetic polymers from cultural heritage surfaces. The ability of five bacteria to attack Paraloid B72, the most commonly used polymer in conservation treatments, was evaluated by optical and scanning electron microscopy observations, weight loss measurements, Fourier transform infrared spectroscopy and differential scanning calorimetric analysis. Although none of the bacteria were able to attack Paraloid B72, the methodology developed can be applied to select other bacteria with this ability. Therefore the results offer insightful guidance to a better design of bioremoval experiments of synthetic resins used in conservation.

1. Introduction

Man-made polymers used to restore art objects have become increasingly important and abundant in museum collections [1]. Indeed, with the aim of preserving artefacts from further chemical–physical deterioration, synthetic polymers have been widely employed as adhesives, consolidants and protective coatings to treat cultural heritage objects [2,3]. Since the 1950s polyacrylates and polymethacrylates, such as Paraloid B72 (PB 72), have been among the polymer-based products frequently used in stone conservation [4].

One of the reasons for introducing synthetic polymers in conservation treatments was the expectation that these materials would be less prone to chemical, physical and biological deterioration than natural organic products [5]. Unfortunately, as the years went by, all the drawbacks of applying such conservation treatment became more evident [2]. Polymer deterioration can modify both the physical properties of the polymer as well as the chemical structure, e.g. via cross-linking or reduction in molecular weight due to chain scission [6,7]. Chemical decay also leads to the formation of oxidized species, quite often producing the yellowing of treated stone surfaces [8], and physical changes induce polymer stiffening and brittleness, often resulting in polymer cracking, detachment from the heritage substratum and worsening of mechanical properties [9]. Although often effective, the application of a synthetic coating on heritage surfaces is usually irreversible, sometimes accelerating, in the long term, the deterioration of the monument or object [10]. Even though it is generally thought that synthetic resins are more resistant to microbial attack than natural organic products, there are many articles in the scientific literature claiming the contrary [11–13]. Meristematic fungi are potential biodeteriogens of outdoor stone consolidated or protected with aged synthetic resins [14]. Moreover, also in indoor environments, synthetic polymers can act as a growth substrate for microorganisms, especially when applied, but not completely removed, during previous or inadequate attempts to restore an artefact [15].

Chemical stability and solubility of synthetic polymers in solvents commonly used in the conservation field are two essential requirements for the reversibility of treatment with synthetic polymers [2,16]. The removal of aged polymeric film from works of art is usually achieved by surfactants and organic solvents (acetone, xylenes, toluene, alcohols), or mixtures of them [17–19]. However exposure to the organic solvents used as cleaning agents in the removal of dirt, stains, overpaints, old varnishes or coatings (e.g. the acrylic resins), can represent a health risk for workers [20]. In fact,

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cleaning agents are potentially toxic, and are often primary irritants of the skin, eyes and mucous membranes [21]. It is also likely that a small part of the solvent used to remove the polymer matrix could be retained in the underlying layer, thus increasing the potential risk to human health [22]. Solvent application can also result in an undesired spreading of the dissolved materials within the porous structure of the artwork [23]. Moreover, cross-linked polymers are very difficult to remove with solvents, although the polymer can be swollen in the solvent and then mechanically removed. After the cross-linking process, it is not possible to employ the same solvent used to apply the undegraded polymer [17]. Recently, microemulsions and micellar solutions have proved effective for the removal of naturally or artificially aged polymeric coatings [23–25]. In contrast with organic solvents, the reduced organic solvent content of microemulsions and micellar solutions lowers the environmental impact of these systems [24] and limits the redistribution of solubilized hydrophobic material [23]. The surfactant content of these systems ranges between less than 1% and c.a. 3–4%, and the surfactant residues after cleaning can be removed from the treated surfaces through accurate washing with water [23–25]. Also cleaning artwork with gels has increased enormously in recent decades. Aqueous, nonaqueous, and mixed gels have been used to remove varnish and overpaint from paint surfaces, and to remove stains from stone. Sequestration of solvents in gel matrices minimizes the deleterious effects of using liquids for cleaning surfaces, and introduces advantages. Moreover, to ensure their complete removal (after the cleaning action) so that no damage occurs to the surface [26], recent significant updates have focused on minimizing or avoiding gel residues after cleaning through the use of peelable gels and chemical gels [27–29].

Biotechnology represents an attractive and sustainable alternative to traditional cleaning in the conservation of cultural heritage materials [30]. Biocleaning methodologies are easily performed, applied and controlled and do not need the presence of skilled and trained personnel [31]. Furthermore, in the case of the biocleaning of a fresco [32] the cost of the biological cleaning using viable bacterial cells was assessed as much lower than that of other conventional methods, making this biotechnology not only very interesting but also very cost competitive. Until now, the bioremediation of undesired organic matter on artwork using living microorganisms has mainly been focused on the removal of casein and animal glues on frescoes [15,32].

The aim of this study was to set up a biological methodology using bacteria for the removal of the naturally-aged synthetic resin Paraloid B72. The ability of five bacterial strains to remove Paraloid B72 by using it as their sole carbon and energy source was evaluated. Since calorimetric [33,34] infrared spectroscopy [35,11] and optical and scanning electron microscopy (SEM) [11] have proved to be very useful techniques in both polymer science and microbiological investigations, we applied these techniques to evaluate the susceptibility of naturally aged Paraloid B72 to bacteria.

2. Material and methods

2.1. Biodegradation assay

Four-year dried Paraloid B72 (PB 72), originally solubilized in 15% ethyl-acetate, was supplied by the Opificio delle Pietre Dure (Florence). The PB 72 was kept in a can under ambient conditions until it was used for the experiments reported in this paper. The resin, a dry layer of 0.5 cm thickness, was cut into seventeen 1×1 cm coupons. The coupons were not sterilized to maintain the chemical and physical characteristics of the material.

Pseudomonas aeruginosa (PA01), *Pseudomonas stutzeri* (ATCC 23856), *Pseudomonas putida* (DeFENS collection, isolated from wastewater treatment plant), *Escherichia coli* (ATCC 25404) and *Bacillus licheniformis* (DeFENS collection, isolated from a biodeteriorated acrylic painting on canvas by a contemporary artist) were the test bacteria used as inocula for biodegradation tests. The bacteria were grown in Trypticasein Soy Broth (TSB, Merck) overnight at 30 °C. The cultures were then aseptically centrifuged three times and the supernatant liquids discarded to avoid any medium residue. The bacterial pellets obtained from each culture were diluted in sterile mineral solution (NaCl 5 g/l and K_2HPO_4 2.5 g/l) to a density of 10^6 cell/ml. The mineral solution, which did not provide any source of carbon, was supplemented with coupons of PB 72 in the presence of and without (negative control) the microbial inoculum. In addition, a positive control was included adding 5% (v/v) TSB as carbon source to the mineral solutions with bacteria. The coupons were placed in Petri dishes and inoculated with 25 ml of the mineral solution supplied with 3% (v/v) microbial inoculum. The Petri dishes were then set to incubate in a climatic chamber at 30 ± 2 °C and 95 ± 5% relative humidity, for 30 days. Each experiment was performed in triplicate. These incubation conditions are not representative of on-site conditions but were used here to favour the bacterial degradation, and therefore make the selection of the most promising bacterium easier. Every ten days after incubation, the microbial activity inside the Petri dishes was checked (data not shown). At the end of the incubation period, polymer solubility was studied with weight loss measurements, polymer surface change with stereomicroscope and scanning electron microscopy observations, and chemical modifications using differential scanning calorimetry (DSC) and Fourier transform infrared spectroscopy (FTIR).

2.2. Polymer characterization techniques

The polymer weight loss induced by the microbial degradation was determined gravimetrically, soaking 0.5 g of the sample in 10 ml of acetone. The specimen was put into the solvent, at constant temperature, and its weight was measured at different times by extracting the sample from the acetone and by removing the solvent wetting the surface with filter paper. The procedure was repeated several times to determine the weight loss. The weight generally decreases over a period of time, depending on the solubility of the macromolecular chains. The evaluation of surface change of the specimens was performed using a stereomicroscope (Wild Heergrugg – Switzerland) and a Field Emission Scanning Electron Microscope (SEM) Zeiss Supra VP40, after metallization of the inoculated and untreated specimens to obtain good conductivity. SEM image acquisition was carried out in Secondary Electron Imaging (SE).

The glass transition temperature (T_g) of the polymers, and its variation during microbial degradation, was evaluated using a Mettler Toledo differential scanning calorimeter (Model DSC 1 STAR^e System) with a heating rate of 20 °C/min from −50 °C to 150 °C. To eliminate any effects deriving from earlier thermal histories, a second heating cycle was always carried out, heating the sample to 150 °C at 20 °C/min and then rapidly cooling it to −50 °C.

Chemical modifications were evaluated by FTIR analysis on a FTIR Bruker Vertex 70 system. The samples were analyzed using Platinum attenuated total reflectance (ATR): a single reflection diamond ATR sampling accessory. Spectra were achieved, between 4000 and 400 cm^{-1}, by the accumulation of 32 scans with the Fourier transformation method, and resolution of 4 cm^{-1}. Spectral acquisitions and data treatments were performed with OPUS software (Version 7.0, Bruker Optics, Inc.).

3. Results and discussion

The potential ability of five bacterial species to degrade a naturally-aged synthetic resin commonly used in conservation