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Fast biocleaning of mediaeval frescoes using viable bacterial cells

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ABSTRACT

The 14th-century fresco *Stories of the Holy Fathers* (size 6.10 × 15.65 m), at Camposanto Monumentale in Pisa, Italy, was painted by Buonamico Buffalmacco. The building housing it was damaged by a bomb during World War II and the fresco was quickly removed from the original walls under extremely dangerous conditions. It was detached using the "tear-off" technique with gauze and a layer of warm animal glue, and it was then stored by rolling it up, without adding any rigid support. In a 1960s restoration, an asbestos-cement support (eternit) was applied to the back and the gauze and thick layer of animal glue were removed from the front of the fresco. Early alteration phenomena such as swelling and detachment of the paint layer were noticed. In 2008, the fresco was again detached from the Camposanto wall for further restoration, which would include removal of residual traces of casein and animal glue from previous restorations and the reattachment of the paintings to a more suitable support. On this fresco, the combined removal in one step of casein and animal glue was based on the fast application of whole, viable bacterial cells of *Pseudomonas stutzeri*, A29 strain, to the fresco surface for a period of 2 h. An assessment of the effectiveness of the biological cleaning test was carried out using analytical pyrolysis. The results confirmed the success of this advanced biological approach for recovering valuable frescoes, and gave insight into selecting the optimum conditions for fast-treatment efficiency. Data on short- and medium-term microbial monitoring confirm both that viable cells are not present in the fresco after biotreatment and the absence of any potentially negative effects that could have been caused by metabolism. The conclusion was that the procedure was safe, non-invasive, and risk-free. The relatively low-cost of this biological cleaning process means that this biotechnological application represents a highly competitive, cost-effective solution.

1. Introduction

The best-known types of artwork, including lithoid materials, stone, frescoes, and paintings, are subject to a number of pathologies, especially when exposed to outdoor environmental conditions. Generally, deterioration is largely due to aging, any historical dirt from nearby fires (wax and smoke) and, in recent decades, to air pollution (Saiz-Jimenez, 1995; Webster and May, 2006). Monuments in urban areas show damage and alterations such as black crust, nitration, sulfation, and the deposition of dust and residual hydrocarbons as a consequence of the large increase in environmental air pollution (Saiz-Jimenez, 1997).

Moreover, when organic matter has been applied, but not completely removed, during previous and/or inadequate attempts at restoration, the surfaces of man-made artistic stonework can also be altered. In these cases, the preservation of the artwork itself poses serious questions (Ranalli et al., 1996, 2000). This type of residual compound, under certain environmental conditions, can act as an adequate growth substrate for microorganisms, namely bacteria and fungi. These can alter and destroy surfaces, resulting in chromatic changes and hyphal penetration (Ranalli et al., 1997; Tiano et al., 2006).

When frescoes are detached from original walls or inadequate support, notable quantities of organic compounds have to be removed prior to restoration. Materials such as animal glue and casein have often been distributed on both surfaces - on the front, as a fixative or consolidator, and on the back of the fresco as an adhesive to the support. The removal of such deteriorated material is one of the main goals in the process of restoration of the frescoes at the Monumental Cemetery in Pisa where they cover a surface area of about 1500 square meters. The frescoes date back to the 14th-century and were painted by Bonamico Buffalmacco, Antonio Veneziano, Benozzo Gozzoli, Taddeo Gaddi, Francesco Traini, and Spinello Aretino. The buildings were badly damaged by a bomb in

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1944, during World War II, and a large number of frescoes were rapidly removed from the original walls under extremely dangerous conditions. They were detached using the "strappo" or "tear-off" technique. This was done using gauze and a layer of warm animal glue, and the frescoes were then stored by rolling them up without adding any rigid support.

Most of the frescoes were restored using both traditional chemical and physical techniques, which are based on the removal of organic residual substances and salts using ammonium carbonate solution and organic solvents. Indeed the enzymes, surfactants, and solubilizing agents used today had the potential to restore some of the frescoes (Makes, 1988; Bellucci and Cremonesi, 1994; Bonomi, 1994; Wolbers, 2007). However, on the basis of pioneering research on the Pisa frescoes, it has been seen that these modern agents fail to restore some frescoes (Ranalli et al., 2005, 2009). In order to solve these problems and to identify suitable restoration practices and methodology, the preliminary approach required specific tests to analyze the original applied organic materials, as well as those from later restoration work.

Subsequently, the materials that had undergone transformation through natural aging and pollution had to be identified and analyzed. For this reason, the use of sensitive, selective, and, if possible, non-invasive techniques that are minimally destructive, such as calorimetry, gas chromatography coupled with mass spectrometry (GC–MS), and pyrolysis/GC–MS, are suggested (Stassi et al., 1998; Colombini et al., 1999, 2003; Bonaduce and Andreotti, 2009).

In our pioneering work on mediaeval frescoes, once the unwanted organic matter on the fresco had been identified, and the difficulty in removing such organic compounds by traditional methods had been verified, we successfully employed viable bacterial cells as "biorestoration" agents. For the first time these biorestoration agents were able to (1) detach the gauze that had been applied during previous restoration and that was adhering to the paint layer on the front surface, thus obscuring the Aretino Spinello fresco, and (2) degrade the organic matter derived from altered animal glue during a 12-h period, followed by complex enzymatic treatment (Ranalli et al., 2003; Antonioli et al., 2005; Cappitelli et al., 2005, 2006, 2007).

In the 1960s, during the first restoration on *Stories of the Holy Fathers* fresco was cut into 17 sections (ranging from 1.3 × 2.0 m to 3.5 × 2.0 m) and attached to an asbestos-cement (eternit) support in order to better continue the restoration. The gauze and a large portion of the animal glue layer on the surface of the fresco were then removed using both traditional chemical and physical techniques, based on the application of ammonium carbonate solution and organic solvents.

Afterward the sections of fresco were re-composed, like a puzzle, on an adequate metal frame at a distance of 50 cm from the walls. This technical solution was adopted in order to guarantee better air circulation from the bottom to the top of the back of the fresco and to reduce the risk of both vertical temperature gradient and of condensation phenomena because of the absence of heating or cooling systems in the main room.

Later, unfortunately, early alteration phenomena, such as swelling and detachment of the painting layer of the frescos, were noticed.

For this reason from 2008 in "our" fresco *Stories of the Holy Fathers*, as happened elsewhere in the Camposanto complex, degradation processes due to the synergism of the organic substances used as adhesive began and sulphation promoted by the lime putty, eternit, and pollution led to the need to again take the frescoes down from the Camposanto walls. Further delicate restoration, which consisted of the removal of residual unwanted organic matter and the reattachment of the paintings to a more suitable support, was performed.

However, during the restoration of this fresco in the 1980s, the main problem encountered had been the difficulty of completely removing all the residual consolidating agents that had been applied. Over the years the proteinaceous materials had caused serious alterations and had become very hard and resistant to commonly used solvents. These included traces of casein from the back of the fresco and diffuse glue residues ranging from a few granules (0.1 mm to about 1 mm thick) to a maximum value of 20 mg/cm^2 on the painted surface.

The state of preservation had also been worsened by the contraction of the residual animal glue and the subsequent swelling of the thin paint layer. Furthermore, different synthetic organic substances had been used in past restorations and these had favored polymerization of some compounds, making their removal difficult.

The aim of the present research was to establish an advanced "fast" biocleaning process based on bio-dissolving to be used in-situ on *Stories of the Holy Fathers* fresco surface, completing full-scale cleaning in a single-step that would remove proteinaceous material residues from past restorations, such as thin layers of animal glue and casein.

The following steps were taken: (1) physical-chemical analysis of the residual adhesive organic matter used in the past to detach the fresco; (2) development and improvement of an advanced biocleaning system to remove this organic matter from the fresco surface; (3) short- and medium-term microbial monitoring; and (4) cost-analysis of the biotechnological system employed and the bacterial cells used.

2. Materials and methods

2.1. Buffalmacco Buonamico fresco description

For the present study the 14th-century fresco *Stories of the Holy Fathers*, painted between 1336 and 1341 by Buffalmacco Buonamico (scene 8, sections 10 and 14) was chosen from the frescoes from the Monumental Cemetery complex in Pisa. The total surface area of the fresco is 95.0 m^2, all of which was biotreated (Fig. 1). The paint contained inorganic pigments, as was already known from the literature. Since the end of 1980s this fresco has been located at the "Camposanto" site, in the "Salone degli affreschi", one of the largest rooms there, which is specially equipped for indoor conservation of these important paintings.

This room is open to visitors through a door leading to a short corridor to the partially open area of the Monumental Cemetery.

2.2. Chemical and physical analyses

2.2.1. Reagents and apparatus

All the solvents used were Baker HPLC grade solvents. Hexadecane and tridecanoic acid were used as internal standards. Hexamethyldisilazane (HMDS) and N,O,bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane were purchased from Sigma (Milan, Italy); N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) with 1% trimethylchlorosilane was from Fluka (USA); and all were used without any further purification. Standard solutions of amino acids in HCl 0.1N, norleucine (I.S.2), and hexadecane (I.S.1) as internal standard injection were purchased from Sigma–Aldrich (USA). Equipment used included a microwave oven, model MLS-1200 MEGA Milestone (FKV, Italy); a CDS 5000 Pyroprobe (CDS Analytical, Inc., Oxford, USA); a 6890N GC system gas chromatograph, (Agilent Technologies-USA), coupled with a 5973 mass selective detector (Agilent Technologies); and a single quadrupole mass spectrometer equipped with split-splitless injector.