BLUE-BIOTECHNOLOGY AND BIOCLEANING OF HISTORIC-ARTISTIC ARTIFACTS

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1. Introduction

Cleaning is frequently one of the first actions in a restoration project. However, it can be seen as going against the principles of conservative restoration as it is an irreversible procedure and an action that may produce a visible change on the artwork surface. Cleaning is undoubtedly able to re-establish the correct reading of a work of art (Figure 1) and is particularly justified if its benefits and the chosen procedure are compatible with the constituent materials and provide the lowest possible risk level for the artifact itself and for restorers' health.

Figure 1. Cleaning, clearly showing re-establishment of the correct reading of the painting surface (by E. La Francesca, Degree in Conservation and Restoration of Cultural Heritage, University of Palermo, Italy).

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The ongoing search for novel strategies continues, others are in the process of being developed, but known strategies are the most appropriate in all restoration procedures. One innovation in cleaning procedures is represented by enzymatic cleaning. Since its action is highly selective, it effectively removes the undesired layers without affecting the constituent or surrounding materials. Proteins with enzymatic activity are hydrolases, which have been tested since the 1970s to remove starch paste, animal glues or protein adhesives and aged acrylic coatings by using purified enzymes as single or mixed solutions [1-4]. Enzymes, such as trypsin, amylase and protease have been used, mainly for the treatment of glue layers on paper, but applications have already been made on a variety of artworks, such as paintings on canvas, mural paintings and wood and stone artworks. Commercial hydrolases, such as proteases, amylases, lipases and esterase are isolated from animal (pancreas, stomach), vegetal (oat and wheat seeds) and microbial (bacteria or fungi) sources and utilized in biocleaning treatments in order to remove specific substrates [5], also in combination with the action of viable bacterial cells [6]. These methods are a very helpful tool in the biocleaning of artworks, often representing a valid alternative to chemical solvents, characterized by selective removal action and safety for both the restorer and the environment [7].

A complete characterization of the surface layer/s to be removed can be performed by Fourier Transform Infrared (FTIR) Spectroscopy [8] or by using biochemical methods such as High Performance Liquid Chromatography (HPLC) [9] or Sodium Dodecyl Sulphate-Polyacrylamide gel (SDS-PAGE) electrophoresis [10].

2. Novel hydrolases (Protease, Esterase)

In the last three years, promising results have been obtained from the collaboration between the Biology and Biotechnology for Cultural Heritage and Immunology laboratories at the University of Palermo. Particularly, cold-active molecules, extracted from marine invertebrate organisms have been utilized in restoration procedures to remove protein layers. Preliminarily, laboratory tests were performed on ad hoc specimens, and only after finding that our hypotheses were established, were the same tests carried out on several artifacts. In a first step, bioactive molecules with protease activity were applied in order to remove protein layers from a wooden artifact, an oil on canvas painting, a wax sculpture [11-14]. Removal efficiency was evaluated by detecting the molecules present on the cotton swabs utilized in the final cleaning procedure, by *Amido Black Staining Solution* [15].

The research focalised on different undesired layers, mainly esters and waxes frequently used in the restoration procedure [16]. Particularly, a set of insect (bee) or vegetal (carnauba) waxes were selected and several related laboratory specimens were assembled; at present the specimens are undergoing a process of artificial ageing (repeated cycles: UV-A 300–400 nm; T = 22 ± 5°C; RH = 60-65%).

In order to set up biocleaning procedures for the removal of these waxes a new set of bioactive molecules was isolated from marine organisms, specifically BME (bioactive molecules with esterase activity). In parallel, we also carried out a cleaning test on Paraloid B72 aged layers, a thermoplastic resin extensively utilized in the field of artwork restoration as adhesive, consolidating agent, filler, etc [17]. In this study, removal of the Paraloid B72 layer was performed on a laboratory specimen

mimicking the "*strappo*" 'of mosaic tesserae, carried out by gluing tesserae on a canvas sheet; this specimen was naturally aged for 5 years (Temp=24±5°C and RU = 58±10%).

Interesting results were achieved after 30 minutes application of bioactive molecules with esterase activity (BME) solution, at 26°C (laboratory environment temperature), as shown in Figure 2.

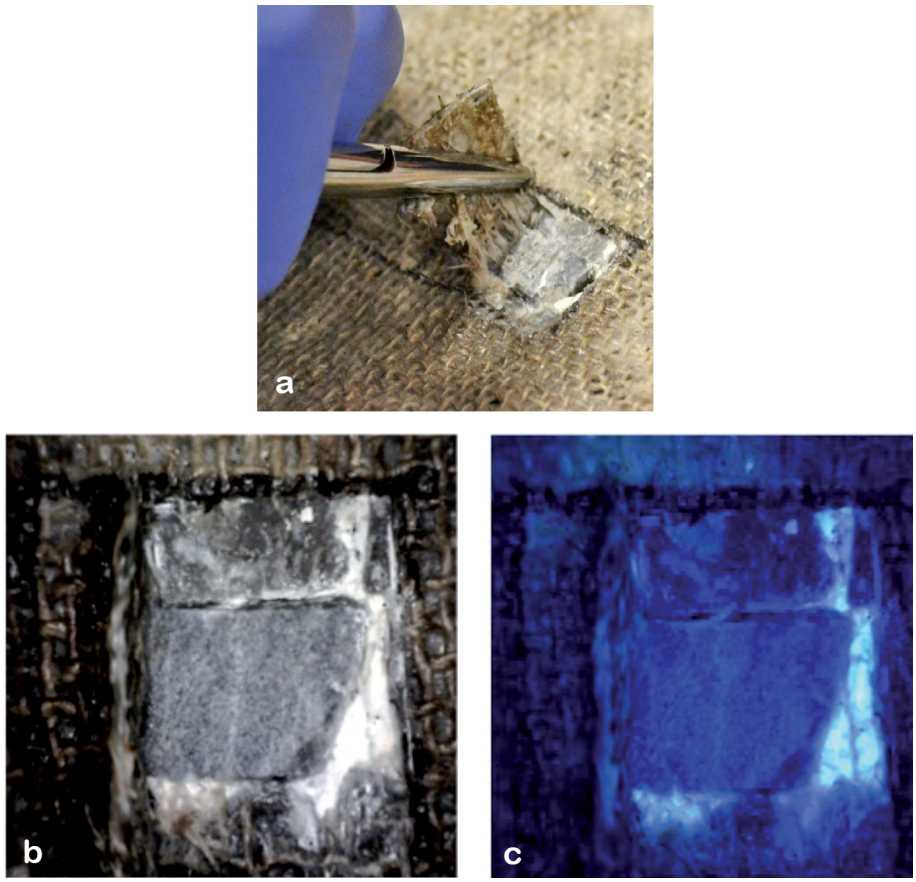

Figure 2. Bio-removal of canvas layer stuck on mosaic tesserae by Paraloid B72: a) after 30 min of application of BME enzyme solution, the canvas layer is removed, but Paraloid residues are still present on the tesserae; b) supplementary application for 10 min of enzyme solution allows complete removal of resin from tesserae surface; c) UV-induced fluorescence support the observation in b).

3. Conclusion

Enzymes are usually utilized in aqueous systems, consequently, they cannot be utilized in all restoration procedures, but can, however, be applied as gelled solutions. Several gelling agents (Klucel-G, Pluronic F108, Vanzan NFC 2) can be utilized as

enzyme support (specific viscosity, water release), which is able to guarantee stable reaction conditions and facilitate the cleaning procedure [13]. Moreover, the enzyme action may be influenced by some inhibitors, found in a large number of ancient and modern pigments, such as heavy metal ions ($Cu^{2+,}$ Pb^{2+}, Cd^{2+}, Hg^{2+}, Sn^{2+}), or by specific pH and temperature values and application time.

Great progress has recently been made in the application of bioactive molecules isolated from marine organisms such as sponges, jellyfish, sea-anemones, shellfish (Blue-Biotechnology) representing an important resource useful to the health, food and processing, and preservation industries. The peculiarities of these molecules are stability, activity at low-temperature (<30°C) and specificity of action. In the field of conservative restoration, the new hydrolase with protease (BMP) or esterase (BME) activity tested in our studies showed selective action avoiding any damage to the constitutive materials. Another peculiarity is the absence of any negative impact on restorers and environment, thus falling into the category of green restoration technologies, indispensable for the sustainable conservation of cultural assets.

Despite the evident positive aspects of the innovative procedure for the biological cleaning of historic-artistic artifacts, a systematic study of how hydrolytic enzymes work on artworks remains to be implemented, with a focus on optimizing the parameters that are crucial for catalytic activity compared to the nature of the undesired layers that must be removed.

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Biographical notes

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