BIOACTIVE MOLECULES: INNOVATIVE CONTRIBUTIONS OF BIOTECHNOLOGY TO THE RESTORATION OF CULTURAL HERITAGE

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1. Introduction

Since the beginning of the twenty-first century, biotechnology has provided innovative techniques useful in the field of diagnosing biodeterioration in cultural heritage and in defining conservation/restoration strategies. From the point of view of diagnosis, molecular biology technology allows sample amounts to be minimised and information in diagnosing microbial contamination of cultural assets to be optimised, thus revealing unknown species that have never been cultured [1-4]. This approach, based on genomic DNA analysis, has also proven to be very useful in revealing and identifying microbial particles in the bioaerosol of indoor environments that may be related to potential health problems in visitors/professional operators [5-7].

From another point of view, science and technology have provided and continue to provide innovative protocols for the biological cleaning (biocleaning) of the surfaces of works of art. It is well known that the action of cleaning represents one of the first and most important steps in a conservative restoration project. It must be performed carefully and in a selective way, making a distinction between different areas, with the aim of preventing irreversible damage to the surface of the object, and removing deposits without acting directly on the original materials of the same surface.

So far many scientific publications have referred that biocleaning, performed by purified enzymes or living bacteria, seems to meet these standards of accuracy. In particular, several bacterial strains, with specific cellular activities, have long been used effectively for remediating polluted environments; in addition, bioreactive molecules (enzymatic protein or enzymes) isolated from different biological systems may be applied to remove overlapping organic layers (proteins, oils, starches) from the surface of historic and artstic works.

The first biocleaning attempts with enzymes date back to 1970 and were initially perfomed on paper-based artifacts and polychrome canvases. However, they were frequently isolated experiences. Wendelbo, reported the enzymatic treatment (trypsin in phosphate buffer at pH 8.0, for 10 minutes at 40° C) on some pages of a book pasted together with animal glue [8]. Segal and Cooper [9] applied a double enzymatic treatment, to remove adhesives composed of starch and protein, using both amylase

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(in phosphate buffer pH 7.0, incubating at 38 °C up to 60 minutes) and protease (in buffer phosphate at pH 7.5 and incubated at 40° C). Makes, in 1982, reported the first application of an amylase/protease mixture to remove glue paste from the back of an oil painting [10] and later, in 1988, the use of a mixture of enzymes to remove a protein/oily binder from a painted surface [11]. Lipases have been applied in different restoration procedures regarding the removal of aged acrylic resins (Paraloid B72) used as a coating [12] or oil-based overpaints, by immobilizing the enzymes in aqueous gel [13]. Enzymatic decoloration of pigmented spots on marble surfaces produced by Serratia bacteria, was performed by the fungal enzyme laccase [14].

An alternative to cleaning with purified enzymes is the use of live bacterial cells, a potential application first perceived by Atlas in 1988, and later implemented by Sorlini, Ranalli and Cappitelli [15-17]. In particular, the application of bacteria *Desulfovibrio desulfuricans* (supported by Carbogel) allowed the removal of black crusts, reducing sulphates to hydrogen sulphide. The same research group performed the bio-cleaning of one of the frescoes in the monumental cemetery of Pisa, using *Pseudomonas* viable bacterial cells (*P. stutzeri*, A29 strain) able to degrade glue layers, minimizing the risk of pigment alteration, reaching an efficiency of 80-90%. The cleaning was performed on different zones of the fresco, keeping the bacteria on the surface for a time between 8-12 hours at 28-30° C. Most of the organic layer was degraded by means of the metabolic activity of the bacteria, while the residual glue was removed by protease (TypeXIX) enzyme solution [18]. In these studies the destructive activity resulting from the metabolism of the bacterial colonies, was successfully used to reduce sulphate in the black crust or hydrolyze animal glue layers. Bioremoval of animal glue from mural paintings, by *P. stutzeri* viable bacteria, has recently been reported by Bosch [19].

2. Potentially useful new enzymes for biocleaning surfaces of works of art

Currently, hydrolases (amylase, protease, esterase) that are commercially available and used for biocleaning surfaces of works of art have been isolated and purified from terrestrial biosystems and applied at temperatures $\geq$ 30° C.

It is possible to isolate amylase from animal tissues or microbial sources (*Bacillus* sp. or *Aspergillus* sp.); lipase derived from animal tissues (pancreas), from plant tissues (seeds of oats and wheat) or from microbial species (*Bacillus*, *Aspergillus*, *Penicillium*). Pepsin, trypsin and protease can be extracted from both animal tissues (pancreas, stomach) and microbial cells (*Bacillus*, *Aspergillus*).

The rational use of a purified enzyme solution or a mixture of them, requires information on: i) the nature of the material to be removed (proteins, starches, oils, fats, waxes); ii) the hydrolytic activity and specificity of the enzyme action; iii) pH and salt concentrations; iv) temperature. Reaction temperature represents a crucial point in biocleaning protocols since it is neither advisable to use preheated (>30°C) enzyme solutions nor to heat the artifact surface to which it must be applied.

In order to avoid undesirable effects on works of art, related to the reaction temperature of the enzyme, in our laboratory we are developing biocleaning protocols involving the use of new hydrolases isolated from marine organisms. Marine organisms are a potentially rich source of different enzymes with unique properties and with a wide range of interesting applications in the field of scientific research and industry. Two batches of hydrolase, with collagenolytic-proteolytic activity were isolated from

vertebrate and invertebrate marine organisms, and the protease activity tested with SDS-PAGE or with the Kembhavi method [20, 21].

The most interesting feature of these enzymes is that they are active in a range of temperatures between 4° C and 37°C.

We have recently performed the removal of protein layers (mainly animal glue) both from ad hoc arranged test specimens (laboratory test) and from polychrome wooden furniture (Museo Diocesano Palermo), selectively and for short lengths of time (5-10 minutes), performed at a temperature of 22±3° C; a range of "room temperature" (19 to 25.5 °C), which coincided with restoration laboratories or storage environments.

The cleaning tests showed reliable results, without the heating of the enzyme solutions or of the surfaces on which they were applied [22, 23]. This particular feature makes these proteases more appropriate than others, usually active at higher temperatures.

3. Conclusions

Our hypothesis is that these enzymes will implement the knowledge and the efficiency of bio-cleaning protocols and can be used to hydrolyze and easily remove animal glue layers that alter the painted surface of artistic works. This feature constitutes an innovation compared to products currently available and is in accordance with modern criteria for restoration: compatibility with the original constituent materials; minimal intervention; actual applicability of the product with a reduction in costs and operating times.

The advantages of using enzymes (or living bacteria) as an alternative to traditional methods lies in the fact that the first generally induce more specific and highly selective processes and are therefore non-invasive. Typically they act only on target compounds, without attacking molecules other than those for which they are used.

Further research, however, is in progress to complete purification and to determine the properties of the single components isolated from invertebrate marine organisms. Moreover, we have also partially purified enzymes with lipolytic activity.

As well as for proteases, lipases represent a valid alternative to conventional methods for removing aged siccative oil, as they represent safer working conditions both as regards the operator's health and the integrity of the artwork.

Moreover, purified enzymes which are already active at temperatures below 30° C, such as those that can be isolated from marine organisms are extremely useful in projects designed for the restoration of historic-artistic works of art.

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Biographical notes

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