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BIOCLEANING OF DAMMAR VARNISH FROM EASEL PAINTINGS: AN EXPERIMENTAL PRELIMINARY APPROACH

Vittoria AVOGARO[1], María del Pilar BOSCH-ROIG[1*], Maria CASTELL AGUSTÍ[1], Patricia SANMARTÍN[2]

[1] Instituto Universitario de Restauración del Patrimonio, Camino de Vera s/n 46022, Universitat Politécnica de València – Valencia, Spain
[2] CRETUS. Gemap (GI-1243), Departamento de Edafoloxía e Química Agrícola, Facultade de Farmacia, Universidade de Santiago de Compostela, 15782 – Santiago de Compostela, Spain

Abstract

Aged varnish layers distort the original appearance or integrity of easel paintings and they must be removed in most cases. Toxic solvents (harmful to conservators, the artwork and the environment) are frequently used. To seek an alternative to these harmful products, here we assay the use of safe bacteria as biocleaning agents. Three bacteria (further here identified as Microbacterium oxydans, Acinetobacter johnsonii and Micrococcus luteus) were isolated from the 18th-century easel painting "The Glorification of Saint Teresa of Jesus by Christ and the Virgin, accompanied by founding saints," School of José Vergara (1726-1799) with damaged Dammar varnish. In the laboratory, the three isolates and a bacterium widely used in biocleaning (Pseudomonas stutzeri) were grown in oligotrophic broth media supplemented with powdered Dammar resin to assess their tolerance and ability to grow using the Dammar resin components as an energy source. Then, the four bacteria were applied with 2% agar-agar to Dammar varnish mook-ups for 1, 2, 3 and 4 hours to test their degradative capacity. The biological treatment efficiency was analyzed by visual examination, grazing light photography, colorimetric and infrared (FTIR) spectroscopy measurements. According to noticeable changes in FTIR spectra, indicating an alteration of the varnish structure, coupled with the presence of bacteria on the surface, P. stutzeri, A. johnsonii and M. luteus are promising for bioremoval of dammar varnish. In addition, naked-eye change assessment showed more intense changes by Acinetobacter johnsonii.

Keywords: *Feasibility study; Resins; Bioremoval; Safety technologies; Cultural heritage; FTIR.*

Introduction

Varnishes have historically been broadly applied in the artistic field to protect the layers of polychromy after drying but also to give them aesthetic values such as brilliance, saturation and depth [1]. Varnishes are applied on a painted surface as a liquid layer that, when it dries, remains as a thin and transparent film, shiny and flexible, which provides luster and protection. Their characteristics depend on the materials used, which can be natural or synthetic resins and have varied in terms of compositional materials throughout history.

Dammar varnish on easel paintings (the object of this study) became common from the mid-19th century onwards and was used as a varnish for paintings or varnishing artworks. This varnish is widely used as a paint contact coating in multilayer varnishes and it is used for the final

* Corresponding author: mabosroi@upvnet.upv.es

protection in conservation and restoration treatments of works generally dated before the middle of the 19th century [2]. It is used due to its great compatibility with the pictorial material and for respecting the synthetic varnishes used as surface coating [2].

Dammar is a resin, generally referring to a group of natural triterpenoid resins exuded from the family Dipterocarpaceae trees [3]. Dammar resin and, in general, triterpenoid resins have been extensively studied and analyzed through different instrumental techniques, but despite this, the macromolecular components of which they are composed are not yet fully understood [3]. Dammar resin generally comprises 62.5% polycadinene, the polymeric part often called resin and 23% resin acids. This resin contains numerous triterpene derivatives, among which dammardienol and hydroxydammarenone are the main ones; the primary acids are dammarenolic acid, oleanolic acid and ursonic acid [3].

Over time, the Dammar varnish layer on easel paintings can suffer physical and chemical degradation, causing discoloration and yellowing. This varnish degradation must be removed because it alters the original artwork's perception and meaning (see e.g., [4]). Chemical products are often used to remove the varnish layers, which are harmful not only to the restorer and the environment but also to the artworks, as it is unknown what effect they will have over time.

Biotechnology represents an attractive and sustainable alternative to traditional cleaning agents for conserving cultural heritage materials. In this sense, biorestoration uses enzymes and live bacteria for bioconsolidation and biocleaning [5]. Enzymes have been used more than live bacteria on paper, canvas and paintings for biocleaning treatments. The first applications of enzymes in restoration interventions dated from the 1970s and were on paper artworks [5-7], applying amylases and proteases (two classes of hydrolases) in gel form or by immersion to remove organic glues or starch adhesives. Later, the use of enzymes was extended to polychrome artworks, being mainly used to remove organic glues, starch adhesives, repaintings (with protein and oil binders), drying oils and acrylic resins such as Paraloid B-72 (see, e.g., [8, 9]). In addition, enzymes (mainly alpha-amylase and, to a lesser extent, protease) have been used to remove animal glues and starch adhesives from ancient textiles (see e.g., [10-12]).

Biocleaning works with enzymes have hardly been carried out on easel paintings. Amylases and proteases were used to remove glue residues of linings, lipases for removing oil-resinous patinas on oil canvas paintings and protein and oil-based overpainting and proteolytic enzymes to remove egg white residues [7]. A few studies have also been done on varnishes. Gelled microbial protease has been used to remove an oil resin varnish from a 15th-century tempera [9]. Bellucci and colleagues reported the elimination of aged Paraloid B-72 with lipase (extracted from *Candida cylindracea*) from two artworks, a 15th-century tempera on panel and a 19th-century oil on canvas [8].

This paper presents an experimental preliminary approach to evaluate the potentiality of four live bacteria (three isolated from an 18th-century oil on canvas painting and one widely used in biocleaning studies) for the biocleaning of altered Dammar varnishes affecting easel paintings. This first attempt represents a promising eco-friendly alternative for removing altered film-forming agents affecting historical easel paintings, which should in the near future be investigated and optimized further.

Experimental part

Materials

The 18th-century oil on canvas painting, “The Glorification of Saint Teresa of Jesus by Christ and the Virgin, accompanied by founding saints,” School of José Vergara (1726-1799),

with altered and aged Dammar varnish, was used to isolate and select putative bacterial strains with Dammar varnish biocleaning potential (Fig. 1). Three microsamples (M1, M2 and M3, of an approximate size of 0.5×0.5mm) were taken with a sterilized scalpel from different areas of the painting (Fig. 1) and placed in Eppendorf tubes to isolate the bacterial strains for the feasibility trials. Eppendorf tubes contained M9 mineral medium (M9 broth (75.2g/L $Na_2HPO_4.2H_2O$, 30g/L KH_2PO_4, 5g/L NaCl, 5g/L NH_4Cl) + $MgSO_4$ (1M) + $CaCl_2$ (1M)) and Nutrient Broth (NB) medium (2g/L yeast extract, 5g/L peptone, 5g/L sodium chloride). Both media were used separately in triplicate for the experiments. Test tubes were incubated for 19 days at 28°C, after which most showed turbidity. Then, to isolate the grown bacteria, 100 µL of each test tube were plated in triplicate on Nutrient Agar (NA, NB plus 15g agar) Petri dishes and incubated at 28ºC for five days. At the end of this period, three morphologically different strains were isolated, one from each of the samples (M1, M2 and M3) taken from the painting. Furthermore and for a comparative purpose, *Pseudomonas stutzeri* CECT 930 was included in the trials, as it is widely used in biocleaning, with good results on paintings (see, e.g., [13]).

Fig. 1. "The Glorification of Saint Teresa of Jesus by Christ and the Virgin, accompanied by founding saints," School of José Vergara (1726-1799). Three red circles indicate the sampling points (micro samples M1, M2 and M3)

To test the potential use of the four target bacteria as Dammar varnish removal agents, Dammar varnish mook-ups were prepared by applying the varnish with a soft brush on regular white ceramic tiles (15cm×15cm) because it is an inert and smooth support, which can also be easily sterilized with alcohol and exposure to UV-C light for 30 minutes.

Methods

Identification of the three bacterial strains isolated by DNA sequencing

Genomic DNA was purified from cell cultures during the exponential growth phase to identify the best-performing strains and stored on Whatman FTA™ Indicating Micro Cards for bacterial isolates by STAB Vida Lda (Portugal). Fragments of the bacterial 16S rDNA gene containing the variable regions V1 to V9 in bacterial samples were PCR amplified using

previously described primers and reported PCR conditions [14]. Sequences were trimmed, edited and checked against the GenBank database by BLAST search, which applied a similarity score of ≥ 99.0% to identify the microorganisms.

Screening assays: Adaptation and growth of the four bacteria with Dammar varnish

The three isolates of bacteria and *Pseudomonas stutzeri* CECT 930 were separately grown with (and thus adapted to) Dammar varnish fragmented and sterilized, following the protocol described by Sanmartín and Bosch-Roig [15] in test tubes of 40mL containing 150µL of inoculated bacterial solution at a concentration of 1.0×10^8 colony-forming units (CFUs)/mL and 20mL of oligotrophic broth medium (M9 mineral medium or Nutrient Broth (NB) medium). Test tubes were incubated at 28°C and checked after 3, 10, 17, 26 and 53 days for visually detectable effects, such as turbidity (indicative of bacterial growth), color change and biofilm formation, recorded at the same times as the CFUs were counted and pH measured. The experiments were performed in quadruplicate. Also, five negative controls (without inoculum, i.e., bacterium) were used for each combination to check the results obtained.

Biocleaning assays with the adapted microorganisms and assessment

Adapted bacteria were applied to Dammar varnish mook-ups prepared to test their degradative capacity. One mock-up for each bacterium was divided into four parts corresponding to the treatment times of 1, 2, 3 and 4 hours each and for each treatment time, there were four replicates. In each replicate, 100µL of bacterial solution at a concentration of 1.0×10^8 colony-forming units (CFUs)/mL was applied and then covered with a 1.8×1.8cm square of cold sterile 2% agar-agar. After each of the test times, a sterilized spatula and tweezers were used to remove the agar. A final cleaning step was done on the surface with a swab and sterile water to remove the bacterial remains completely.

Physical changes visible to the naked eye following biological treatment were evaluated and photographed with a Nikon D300 digital camera. Hereafter, color changes were quantified using a Minolta CM2600D colorimeter and the CIELAB color system [16], which represents each color using three coordinates: L*, lightness of color, which varies from 0 (absolute black) to 100 (absolute white); a*, associated with changes in redness–greenness (positive a* is red and negative a* is green) and b*, associated with changes in yellowness–blueness (positive b* is yellow and negative b* is blue). Three color measurements (taking the coordinates L*, a* and b*) were done on each replicate before and after the bacterial treatment. The total color difference (ΔE^*_{ab}) was also calculated as follows: $\Delta E^*_{ab} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$, where $\Delta L^* = L^*_f - L^*_0$; $\Delta a^* = a^*_f - a^*_0$ and $\Delta b^* = b^*_f - b^*_{0;\,f}$ denotes the end of the experiment and $_0$ denotes the beginning of the experiment.

Physical and chemical changes in the Dammar varnish and the presence of the selected bacteria were evaluated for infrared (FTIR) spectroscopy analysis. The ALPHA II spectrophotometer and the OPUS spectra analysis software were used to monitor the changes in the Dammar resin before and after using the four bacteria.

Results and discussion

Target bacteria isolated

From each of the three samples (M1, M2 and M3), a morphologically different colony was isolated. The isolated colonies were successfully identified, revealing bacterial DNA sequences that were identified as belonging to *Microbacterium oxydans* (100% similarity in nucleotide identity with accessions MK942422.1) isolated from M1, *Acinetobacter johnsonii* (99.77% similarity in nucleotide identity with accessions MN826586.1) isolated from M2 and

Micrococcus luteus (99.85% similarity in nucleotide identity with accessions MN826453.1) isolated from M3.

Microbacterium oxydans [17] is a gram-positive bacterium with a bacilli shape that does not form spores or show motility and colonies show yellow pigmentation on nutrient agar. They have aerobic metabolism and can hydrolyze gelatin and casein [18]. Studies have also reported their use in treating heavy metal waste and for reusing the remains of brown seaweed [19].

Acinetobacter johnsonii is a gram-negative bacterium with a short bacilli shape in the growth phase, becoming more colloid or spherical in the stationary phase. They are arranged in pairs, chains or irregular groups and can vary in staining, size and arrangement. *A. johnsonii* is non-motile, does not form spores and is an obligate aerobic bacterium. It is widely distributed in nature, with water and soil being their main ecological niches. Most strains do not reduce nitrates to nitrites and use various organic compounds as a carbon source [20]. *A. johnsonii* has been described as a carbonatogenic bacterium commonly found in Mediterranean calcareous rocks and can induce calcium carbonate precipitation in deteriorated stones [21, 22]. Also, in calcareous cave walls, a close relationship between its metabolism and the calcification process has been proven [22].

Micrococcus luteus is a gram-positive bacterium with a coccoid shape, yellowish pigment, non-motility and obligate aerobic. It is widely distributed in nature, found in soil, dust, water, air (commonly found on environmental monitoring plates) and even in mammalian skin and humans. *M. luteus* is known to colonize textiles and has been isolated from historical objects, such as bricks, wood, oil paintings, linen and papyrus masks (see, e.g., [23]). *M. luteus* has been identified as a cause of biodeterioration in oil paintings [24, 25]. Gupta and colleagues [26] used a strain of *M. luteus* for the degradation of high-density polyethylene (HDPE), obtaining promising results given the bacteria's efficient biofilm-forming capacity and potential for degradation of this material.

Lastly, the bacterium chosen because it is widely used in biocleaning, with good results on paintings [13, 15], *Pseudomonas stutzeri* is a gram-negative bacterium, rod-shaped, that does not form spores and is widely distributed in the environment.

Screening assay results of the four target bacteria are presented in Table 1. Only in the Nutrient Broth (NB) medium and not in the M9 mineral medium, did the four bacteria start to grow from the 3rd day of culture onward. They achieved high growth, an increase of turbidity and biofilm formation from the 10th day of culture onward (Table 1 and Fig. 2).

Table 1. Screening assay results over a 53-day growth period. Sample code: D: Dammar varnish + P: *Pseudomonas stutzeri*, M: *Microbacterium oxydans*, A: *Acinetobacter johnsonii*, ML: *Micrococcus luteus* + M9: mineral medium, NB: Nutrient Broth medium. Result code: f = film of M9 salts, T: turbidity, B = biofilm formation - = absence of bacterium, + = moderate bacterium growth (countable plate count colonies <300CFU ml^{-1}); ++ = high bacterium growth (uncountable plate count colonies >300CFU ml^{-1})

	Incubation Period (days)				
Sample	**3**	**10**	**17**	**26**	**53**
DPM9	-f	-f	-f	-f	-f
DMM9	-f	-f	-f	-f	-f
DAM9	-f	-f	-f	-f	-f
DMLM9	-f	-f	-f	-f	-f
DPNB	+TB	++TB	++TB	++TB	++TB
DMNB	+TB	++TB	++TB	++TB	++TB
DANB	+TB	++TB	++TB	++TB	++TB
DMLNB	+TB	++TB	++TB	++TB	++TB

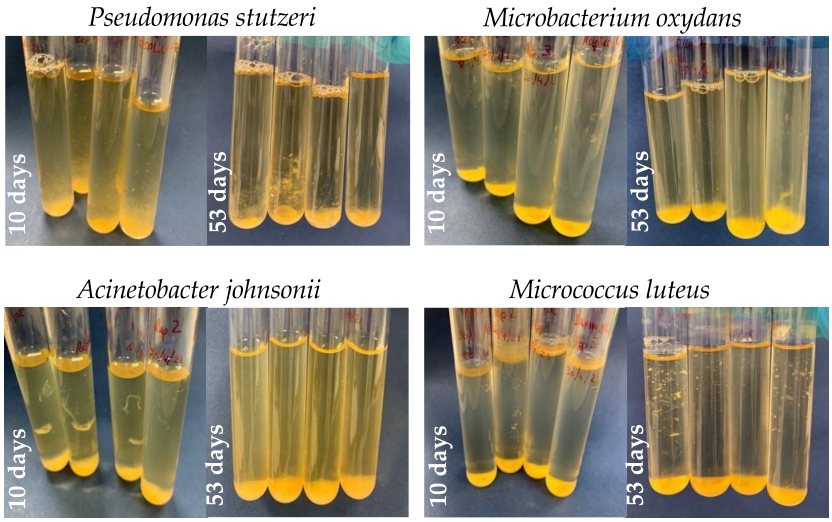

Fig. 2. Test tubes with NB medium over 10-day and 53-day growth periods show the same appearance

Between the 3rd and 10th day, the pH increased from 6-7 to 7-8. None of the four bacteria incubated in M9 was able to grow in 53 days, showing an absence of turbidity in all the replicas. The only visually detectable effect throughout the experiment was an M9 broth salt film on the Dammar resin fragments. The absence of bacterial growth and a salt film were also present in the controls of M9 (without bacterium). No pH changes were recorded in the M9 tests.

Biocleaning tests evaluation

Figure 3 shows the visually detectable effects of the biological treatment, which were observable by the naked eye, provoked by the four bacteria and compared with the control without bacteria.

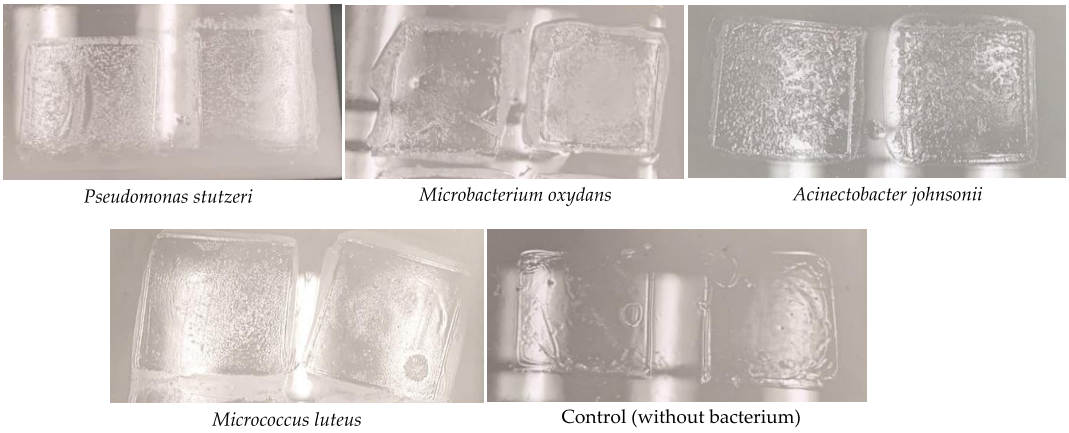

Fig. 3. Dammar varnish samples after 4 hours of bacterial treatment

Visible changes in pinhole formation occurred mainly in the resin treated with Acinetobacter johnsonii, which led to a bigger size hole formation, suggesting the highest biodeterioration capacity. *Pseudomonas stutzeri* and *Micrococcus luteus* also showed some, but minor, alteration, while *Microbacterium oxydans* had a very similar result to the bacteria-free control.

Color measurements were carried out on the same samples to ensure no detectable color change due to the effect of bacteria, as the aim is to eliminate the Dammar varnish but not to introduce side effects. Table 2 shows the partial (ΔL*, Δa* and Δb*) and total (ΔE^*_{ab}) color differences. According to colorimetry criteria, a color difference between 0.73 and 1 CIELAB units is an approximate threshold or just a noticeable difference (jnd), constituting the lower perception limit in an individual with normal color vision [27]. Table 2 shows that very few samples reach this minimum noticeable color change limit. In addition, the overall low value of the color differences does not follow a logical pattern, with the color change sometimes being greater after less than after more hours of treatment. These results indicate that the treatment leaves no residue and does not alter the color, so it can be considered an innocuous treatment.

Table 2. Partial (ΔL*, Δa*, Δb*, CIELAB units) and total (ΔE^*_{ab}, CIELAB units) color differences after bacterial treatment. Differences above the threshold of 0.73 CIELAB units are shown in bold and underlined

		Time of application															
		1h				**2h**				**3h**				**4h**			
		Replica of the sampling point															
		1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
P. stutzeri	**ΔL***	0.44	0.72	0.33	0.44	0.65	0.27	0.67	0.46	0.52	0.43	0.22	0.55	0.12	0.70	0.47	0.38
	Δa*	-0.16	- 0.14	-0.14	-0.17	-0.15	-0.11	-0.17	-0.13	-0.21	-0.16	-0.20	-0.14	-0.11	-0.11	-0.10	-0.08
	Δb*	0.01	-0.16	0.05	0.01	-0.12	-0.12	-0.14	-0.10	-0.08	0.06	0.05	-0.08	0.07	-0.19	-0.07	-0.05
	ΔE^*_{ab}	0.47	**0.75**	0.36	0.47	0.68	0.31	0.70	0.49	0.57	0.46	0.30	0.57	0.18	**0.73**	0.49	0.39
M. oxydans	**ΔL***	0.30	0.56	0.28	0.34	0.34	**0.73**	0.37	0.45	**0.85**	0.47	0.49	0.46	0.14	0.31	0.53	-0.04
	Δa*	-0.05	-0.07	-0.06	-0.09	-0.06	-0.06	-0.10	-0.12	-0.08	-0.09	-0.07	-0.09	-0.13	-0.13	-0.10	-0.09
	Δb*	0.06	-0.12	-0.09	-0.01	0.00	-0.15	0.06	-0.03	-0.31	-0.02	-0.17	-0.04	0.06	0.03	-0.02	0.10
	ΔE^*_{ab}	0.31	0.58	0.30	0.35	0.34	**0.75**	0.39	0.46	**0.91**	0.48	0.52	0.47	0.20	0.34	0.54	0.14
A. johnsonii	**ΔL***	0.26	0.46	0.45	0.33	0.36	0.43	0.51	0.31	0.33	0.24	0.47	0.27	0.59	-0.27	0.50	-0.06
	Δa*	-0.11	-0.11	-0.12	-0.11	-0.11	-0.11	-0.10	-0.12	-0.11	-0.12	-0.11	-0.09	-0.10	-0.22	-0.04	-0.12
	Δb*	0.10	-0.01	-0.04	0.00	-0.04	-0.04	0.01	-0.05	-0.05	0.00	-0.18	-0.05	-0.16	0.65	-0.39	-0.06
	ΔE^*_{ab}	0.30	0.47	0.47	0.34	0.38	0.45	0.52	0.33	0.35	0.27	0.52	0.29	0.62	**0.74**	0.71	0.15
M. luteus	**ΔL***	0.52	0.28	0.56	0.64	0.59	0.32	**0.83**	0.57	**0.94**	0.65	0.42	0.45	0.42	0.30	0.50	0.29
	Δa*	-0.08	-0.10	-0.13	-0.11	-0.10	-0.10	-0.13	-0.05	-0.11	-0.12	-0.11	-0.12	-0.10	-0.11	-0.12	-0.12
	Δb*	-0.23	-0.06	-0.09	-0.16	-0.17	-0.08	-0.09	-0.13	-0.21	0.01	-0.17	-0.02	-0.02	-0.02	-0.09	-0.04
	ΔE^*_{ab}	0.58	0.30	0.58	0.67	0.62	0.35	**0.84**	0.59	**0.97**	0.66	0.46	0.47	0.43	0.32	0.52	0.32

FTIR spectra of the Dammar varnish before (control) and after 1, 2, 3 and 4 hours of bacterial treatment are shown in figure 4.

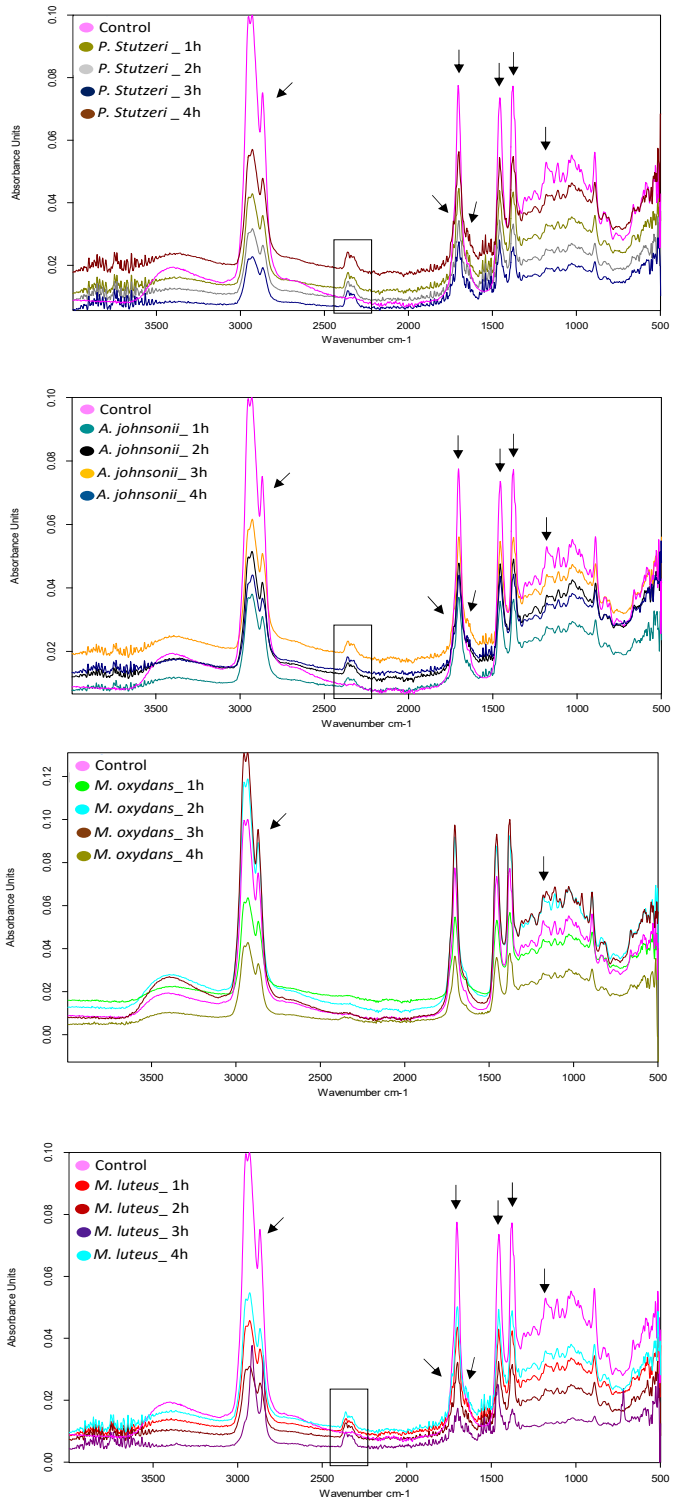

Fig. 4. FTIR spectra of the dammar varnish samples. The arrows indicate the characteristic peaks of the dammar varnish used to track bacterium-induced changes and the squares indicate bands associated with bacterium presence

The spectrum of the dammar varnish shows an intense absorption band at 1713cm^{-1}, assigned to stretching vibrations of C=O groups from aldehydes, ketones and carboxylic acids [28], which decreased in intensity and a shoulder, which increased in intensity with increasing the hours of treatment, at about 1730cm^{-1} can also be observed in the spectra of Dammar varnishes treated with *Pseudomonas stutzeri*, *Acinetobacter johnsonii* and *Micrococcus luteus.* These changes are associated with a degradation of the resin. The structural degradation of dammar resin was evidenced by the decrease of the bands at 1459 and 1379cm^{-1}, which imply a reduction of the C–H bending vibration modes of methyl and methylene species. The two bands at 1180 and 1154cm^{-1} assigned to saturated C–C stretching vibrations decrease in intensity, suggesting triterpenoid scissions during degradation. Bands near 2342 and 2360cm^{-1}, related to amino acids and phosphorus compounds, such as the absorbance peak at 2358cm^{-1} that illustrates the -C=N stretching, are related to the presence of bacterial biofilm on the Dammar varnish sample. According to the FTIR results, only three tested bacteria have potential biodegradation capacity, leaving out *Microbacterium oxydans*.

Conclusions

Here, we conducted a feasibility study involving the search for live bacteria capable of degrading Dammar varnish. The study constitutes a first approach to the biocleaning of Dammar varnish with the use of microorganisms and a step forward towards extending the use of bacteria in removing materials from works of art that have lost their properties, making their removal necessary.

The use of sustainable and non-toxic materials has been making its way in the field of conservation and restoration of cultural property thanks to the awareness of restorers, not only in terms of health but also in terms of all those residues that can affect the environment and the artwork. The use of microorganisms is an option that needs further research on its possible uses and a tool that can be valuable in the path towards more sustainable interventions, extending its use increasingly also to organic artworks.

Based on the tests carried out and the results obtained, biocleaning could be effective in the removal of Dammar varnish using *Pseudomonas stutzeri*, *Acinetobacter johnsonii* and *Micrococcus luteus*, which led to alteration of the varnish structure and no appreciable color change due to treatment with bacteria. In addition, the naked eye change assessment showed more intense changes by *Acinetobacter johnsonii,* making it the one with the highest potential.

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References

[1] A. Calvo, **Conservación y Restauración. Materiales, técnicas y procedimientos de la A a la Z,** El Serbal, La Rioja, 2006, p. 36.

[2] A. Phenix, J. Townsend, *A Brief Survey of Historical Varnishes*, **Conservation of Easel Paintings**, Routledge: Abingdon, UK, 2012, pp. 252-263.

[3] I. Bonaduce, F. Di Girolamo, I. Corsi, I. Degano, M.R. Tinè, M.P. Colombini, *Terpenoid Oligomers of Dammar Resin*, **Journal of Natural Products**, **79**(4), 2016, pp. 845-856, https://doi.org/10.1021/acs.jnatprod.5b00916.

[4] A. Buder, *Dammar resin degradation revisited*, **CHIMIA International Journal for Chemistry, 62**(11), 2008, pp. 913-917, https://doi.org/10.2533/chimia.2008.913.

[5] I. Soffritti, M. D'Accolti, L. Lanzoni, A. Volta, M. Bisi, S. Mazzacane, E. Caselli, *The potential use of microorganisms as restorative agents: an update*, **Sustainability**, **11**(14), 2019, Artice number: 3853, https://doi.org/10.3390/su11143853.

[6] I. Castiñeyra, S. Ferrer, M. Moya, *Fundamentos y antecedentes de la utilización de enzimas en tratamientos de limpieza,* **Revista PH, 53**, 2005, pp. 24-34, https://doi.org/10.33349/2005.53.1966.

[7] P. Cremonesi, A. Casoli, *Enzymes as tools for conservation of works of art***, Journal of Cultural Heritage, 50**, 2021, pp. 73-87, https://doi.org/10.1016/j.culher.2021.06.005.

[8] R. Bellucci, P. Cremonesi, G. Pignagnoli, *A preliminary note on the use of enzymes in conservation: the removal of aged acrylic resin coatings with lipase*, **Studies in Conservation, 44**(4), 1999, pp. 278-281, https://doi.org/10.2307/1506657.

[9] N. Buttazzoni, A. Casoli, P. Cremonesi, P. Rossi, *Preparazione e utilizzo di gel enzimatici, reagenti per la pulitura di opere policrome*, **Progetto Restauri**, **7**(16), 2000, pp. 11-19.

[10] H.E. Ahmed, F.N. Kolisis, *An investigation into the removal of starch paste adhesives from historical textiles by using the enzyme α-amylase*, **Journal of Cultural Heritage, 12**(2), 2011, pp. 169-179, https://doi.org/10.1016/j.culher.2010.08.001.

[11] M. Ferrari, R. Mazzoli, S. Morales, M. Fedi, L. Liccioli, A. Piccirillo, T. Cavaleri, C. Oliva, P. Gallo, M. Borla, M. Cardinali, E. Pessione, *Enzymatic laundry for old clothes: immobilized alpha-amylase from Bacillus sp. for the biocleaning of an ancient Coptic tunic*, **Applied Microbiology and Biotechnology, 101**(18), 2017, pp. 7041-7052, https://doi.org/10.1007/s00253-017-8437-8.

[12] H.E. Ahmed, F.N. Kolisis, *A study on using of protease for removal of animal glue adhesive in textile conservation*, **Journal of Applied Polymer Science, 124**(5), 2012, pp. 3565-3576, https://doi.org/10.1002/app.34053.

[13] M.P. Bosch-Roig, J.L. Regidor-Ros, M.P. Soriano-Sancho, R.M. Montes-Estellés, *Biocleaning of animal glue on wall paintings by Pseudomonas stutzeri*, **Chimica Oggi – Chemistry Today, 31**(1), 2013, pp. 50-53.

[14] P. Sanmartín, R. Carballeira, *Changes in heterotrophic microbial communities induced by biocidal treatments in the Monastery of San Martiño Pinario (Santiago de Compostela, NW Spain)*, **International Biodeterioration and Biodegradation**, **156**, 2021, Article number: 105130, https://doi.org/10.1016/j.ibiod.2020.105130.

[15] P. Sanmartín, P. Bosch-Roig, *Biocleaning to remove graffiti: a real possibility? advances towards a complete protocol of action*, **Coatings**, **9**(2), 2019, Article number: 104, https://doi.org/10.3390/coatings9020104.

[16] * * *, **CIE, 1986. Publication 15–2: Colorimetry**. CIE Central Bureau, Vienna.

[17] P. Schumann, F.A. Rainey, J. Burghardt, E. Stackebrandt, N. Weiss, *Reclassification of Brevibacterium oxydans (Chatelain and Second 1966) as Microbacterium oxydans comb. nov*., **International Journal of Systematic and Evolutionary Microbiology**, **49**, 1999, pp. 175-177, https://doi.org/10.1099/00207713-49-1-175.

[18] K. Laffineur, V. Avesani, G. Cornu, J. Charlier, M. Janssens, G. Wauters, M. Delmée, *Bacteremia due to a novel Microbacterium species in a patient with leukemia and description of Microbacterium paraoxydans sp. nov.,* **Journal of Clinical Microbiology, 41**(5), 2003, pp. 2242-2246, https://doi.org/10.1128/jcm.41.5.2242-2246.2003.

[19] M. Gómez-Ramírez, L.A. Montero-Álvarez, A. Tobón-Avilés, G. Fierros-Romero, N.G. Rojas-Avelizapa, *Microbacterium oxydans and Microbacterium liquefaciens: a biological alternative for the treatment of Ni-V-containing waste*, **Journal of Environmental Science and Health. Part A Toxic/Hazardous Substances and Environmental Engineering, 50**(6), 2015, pp. 602-610, https://doi.org/10.1080/10934529.2015.994953.

[20] T.D.H. Bugg, *Dioxygenase Enzymes and Oxidative Cleavage Pathways,* **Comprehensive Natural Products III (Third Edition), Chemistry and Biology, 5**, 2010, pp. 137-172, https://doi.org/10.1016/B978-0-08-102690-8.00169-X.

[21] P. Kämpfer, *Acinetobacter,* **Encyclopedia of Food Microbiology, Second Edition**, 2014, pp. 11-17.

[22] S. Banerjee, S. Joshi, *Ultrastructural analysis of calcite crystal patterns formed by biofilm bacteria associated with cave speleothems*, **Journal of Microscopy and Ultrastructure, 2**(4), 2014, pp. 217-223, https://doi.org/10.1016/j.jmau.2014.06.001.

[23] K. Pietrzak, M. Puchalski, A. Otlewska, H. Wrzosek, P. Guiamet, M. Piotrowska, B. Gutarowska, *Microbial diversity of pre-Columbian archaeological textiles and the effect of silver nanoparticles misting disinfection*, **Journal of Cultural Heritage, 23**, 2017, pp. 138-147, https://doi.org/10.1016/j.culher.2016.07.007.

[24] A.M. Seves, S. Sora, O. Ciferri, *The microbial colonization of oil paintings. A laboratory investigation*, **International Biodeterioration and Biodegradation**, **37**(3-4), 1996, pp. 215-224, https://doi.org/10.1016/S0964-8305(96)00006-6.

[25] F. Troiano, S. Vicini, E. Gioventù, P.F. Lorenzi, C.M. Improta, F. Cappitelli, *A methodology to select bacteria able to remove synthetic polymers*, **Polymer Degradation and Stability, 107**, 2014, pp. 321-327, https://doi.org/10.1016/j.polymdegradstab.2013.12.029.

[26] K.K. Gupta, K.K. Sharma, H. Chandra, *Micrococcus luteus strain CGK112 isolated from cow dung demonstrated efficient biofilm-forming ability and degradation potential toward high-density polyethylene (HDPE)*, **Archives of Microbiology**, **204**, 2022, Article number: 402, https://doi.org/10.1007/s00203-022-03023-4.

[27] M. Melgosa, M.M. Pérez, A. Yebra, R. Huertas, E. Hita, *Algunas reflexiones y recientes recomendaciones internacionales sobre evaluaci´on de diferencias de color*, **Óptica Pura y Aplicada, 34**(1), 2001, pp. 1-10.

[28] C.-M. Popescu, C. Vasile, B. Simionescu, *Spectral characterization of natural resins used in conservation*, **Revue Roumaine de Chimie (Roumanian Journal of Chemistry)**, **57**, 2012, pp. 495-499.

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