Omics & Heritage Workshop

May 14-15, 2024 - Rome, Italy
Sala Marconi, CNR
Piazzale Aldo Moro, 7 - 00185 Rome

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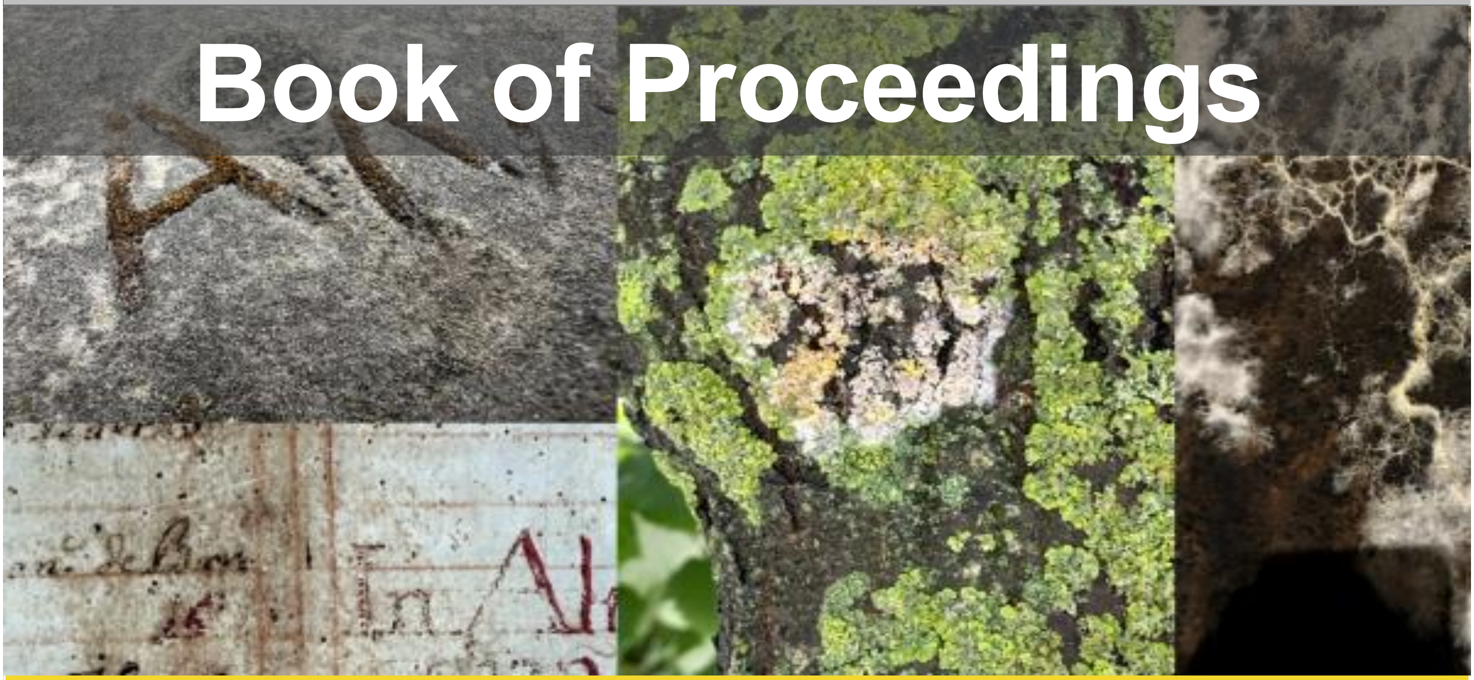
Metagenomes and Microbiomes for the study of Cultural Heritage Conservation and Archaeology

Book of Proceedings



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Programme

Tuesday, 14 May 2024

13:00-13:30 Registration

Aula Marconi, National Research Council of Italy (CNR), Piazzale Aldo Moro, 7 Roma

13:30-14:00 Welcome Address

- Zeineb Aturki, Flavia Pinzari - Institute for Biological Systems (IBS), National Research Council of Italy (CNR)
- Teresa Rinaldi - Dept. of Biology and Biotechnologies Charles Darwin, Sapienza University of Rome
- Massimo Reverberi - Dept. of Environmental Biology, Sapienza University of Rome

14:00-14:30 Keynote Speaker

- Matthew Collins (Department of Archaeology, University of Cambridge, Museum of Natural History, University of Copenhagen): “Skin deep? What can we learn about manuscripts from analysing their biomolecular record?”

14:30-15:10 Session I: Metagenomics and Metabolomics for Archeology

(Chairs: M. Collins and M. Beccaccioli)

- Alessia Monticone: "Omics at the Musei Reali di Torino. Zooarchaeology by Mass Spectrometry (ZooMS) for taxonomic identification of osseous objects and the detection of restoration glues from legacy collections”
- Ylenia Vassallo: “Nanopore sequencing for biocodicology: a new strategy to study DNA in parchment material”

15:10-15:50 Coffee break

15:50-16:50 Session II: Molecular and Microbiological Methods in Preservation of Cultural Heritage

(Chairs: T. Rinaldi and Y. Vassallo)

- Patrycja Petrasz: “A novel mechanism for archaeological iron conservation using dead biomass of the yeast Meyerozyma sp.”
- Cristina Cattò: “Selection and identification of indigenous microorganisms living on spray painted surfaces to be used for cleaning graffiti”
- Adele Bosi: “PARCA project - advance in proteomics and analysis of dyes and recovery of charred and aged textiles”

16:50-17:20 Invited Speaker

- Allegra Via (ELIXIR Italy Training Coordinator, Dept. of Biochemical Sciences "A. Rossi Fanelli, Sapienza University of Rome): "Empowering the microbiome community through the ELIXIR Italy life sciences infrastructure"

17:20-18:00 Flash talks

- Marco Curione: "A proposal for a green selective chemical cleaning method of paper artifacts"
- Giulia Gasperuzzo: "Extraction and characterisation of microalgae-based polysaccharides and protein mixture: potential and innovative material for paper conservation"
- Matilde Kratter: "Evaluation of biodeterioration in the UNESCO site Etruscan Necropolis of Tarquinia"
- Maria-Antonietta Buccheri: "Microbial communities inhabiting deteriorated frescoes: a metagenomic approach to the case of Santa Maria della Grotta"
- Luigi, Faino: "Advantages in applying long reads metabarcoding in cultural heritage conservation"
- Syeda Fatima Manzelat: "Biodeterioration and Biodegradation of Historic Parks of UK by Algae"
- Franco Palla: "Integrated approach to Identify and Counteract Microbial deterioration of Cultural Asset"

18:00-19:00 Poster Session & Drinks

Gypsoteca, Department of Classics (CU003), Sapienza University of Rome, Piazzale Aldo Moro, 5 Rome

19:00-21:00 Dinner

Gypsoteca, Department of Classics (CU003), Sapienza University of Rome, Piazzale Aldo Moro, 5 Rome

Wednesday, 15 May 2024

09:00-09:15 Invited Speaker

- Costanza Miliani (Director of the CNR-ISPC (Institute of Heritage Science): "The E-RIHS IP Project: European Research Infrastructure for Heritage Science Implementation Phase"

09:15-09:30 Introduction to sponsors and publishing opportunities

- Flavia Pinzari
  - The International Biodeterioration and Biodegradation Society (IBBS)
  - The Journal for Applied Microbiology (JAM)
  - The Applied Microbiology International Society (AMI)
  - Sara Envimob Srl

09:30-10:00 Keynote Speaker

- Guadalupe Piñar (Institute for Natural Sciences and Technology in the Arts, Academy of Fine Arts Vienna): "Omic analyses using Nanopore technology applied to the conservation and monitoring of cultural heritage: is this the future?"

10:00-11:00 Session III: Metagenomics and Metabolomics for Heritage Conservation

(Chairs: G. Piñar and M. Beccaccioli)

- Chiara Alisi: "Unveiling the microbial hazard: exploring biofilm composition on Casino Algardi for conservation insight"
- Domenico Celi: "Microbiological air monitoring and biodeterioration risk in the Cathedral of Santa Maria del Fiore"
- Brunella Cipolletta: "Binder-pigment interaction by proteomic approaches"

11:10-11:30 Coffee break

11:30-13:10 Session IV: New and Green Molecules in Preservation of Cultural Heritage

(Chairs: F. Pinzari and L. Pin)

- Luis Fernandes: "Analysis of the fungal communities contaminating museum collections and the fungicidal effects of hydrodistilled plant essential oils: a case study in the Science Museum of the University Coimbra"
- Jelena Pavlovic: "MinION sequencing: taxonomical and functional genes identification of microbiota colonising cultural heritage objects"
- Roberta Ranaldi: "New conservation techniques based on essential oils encapsulated in an alginate hydrogel system to counteract biofilm growth in the Colosseum Arena"

- Filippo Pasquale Riggio: "Carotenoid-producing Actinomycetota associated with pink patinas on ancient mural paintings"
- Brunella Perito: "Essential oils to control microbial growth on the external marble of Florence Cathedral"

13:10-13:20 Announcement of award winners for best poster, presentation and flash talk

13:20-13:30 Conclusions and Future Perspectives

Unveiling the microbial hazard: exploring biofilm composition on Casino Algardi for conservation insights

[1]***Alisi, C.***, [2]Fonti, V., [1]Martinelli M., [1]Migliore, G., [1]Paganin, P., & [1]Flavia Tasso

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The presence of microbial biofilms on buildings and historical monuments can cause both aesthetic and chemical-physical damage: discoloration, corrosion from biogenic acids, crystallisation of secondary soluble salts, and physical penetration. Knowledge of the biodiversity of the organisms that compose these biofilms can help control their expansion and the consequent stone degradation. To formulate effective conservation strategies to prevent and/or combat biodeterioration, it is therefore important to identify the types of microorganisms and their action on the substrate. This study aims to document, by NGS sequencing, the microbial communities that compose the biofilms present on the external walls of the Casino Algardi (Rome, Italy), located in an urban park and never previously studied from a microbiological point of view. The resulting data provide a snapshot of the microbiomes inhabiting the monument at the time of sampling, serving as a baseline for ongoing assessment of the natural biocide's efficacy, a liquorice extract, applied during conservation interventions.

Total genomic DNA was extracted from the biofilm samples and high-throughput phylogenetic 16S and ITS rRNA gene analysis allowed the assessment of the composition of the microbial biofilms potentially responsible for the biodeterioration of the stone facade. The presence/absence of the different populations in the areas under investigation seems to mainly depend, apart the seasonality of the sampling, on the distance from the ground. Apparently, the bioreceptivity of the different materials (plaster or travertine) plays a secondary or completely negligible role in determining the composition of the biofilm.

Structural study of Carbonic Anhydrases for bioconsolidation applications

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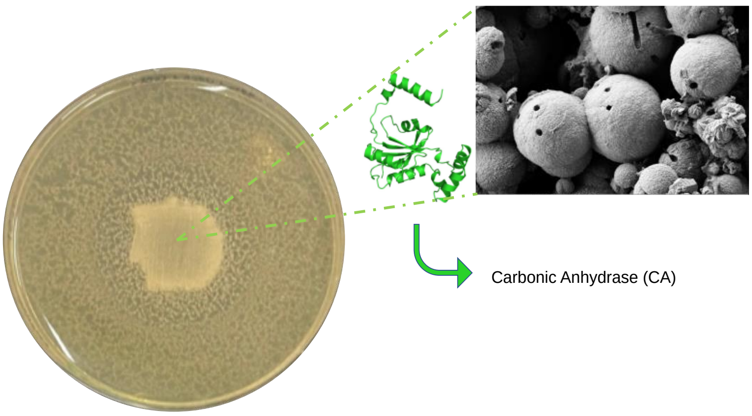
[#]Benedetti, F. and Cirigliano, A. contributed equally.

Microbial-induced calcium carbonate precipitation (MICCP) is a naturally occurring biological process in which bacteria promote the production of calcium carbonate, from microscopic crystals to large geological formations. This technology has been extensively explored in various environmentally friendly applications, such as the biorestoration of limestone monuments and carbon sequestration by mineral carbonation (1,2). An important role in the microbially induced calcium carbonate precipitation process is played by carbonic anhydrase (CA), a key enzyme present in all living organisms (3,4,5). This zinc-containing protein, which facilitates the interconversion of CO2 and bicarbonate, has a wide distribution and participates in many physiological processes, such as cellular pH regulation, and acid and ion transport. By analysing the ability to precipitate calcium carbonate crystals, we selected the most suitable bacterial strains for bioconsolidation applications. To gain insights into the structure-function relationships, the CAs of the different isolated strains were identified and classified from the structural point of view. A comprehensive comparative study led to the identification of the structural determinants of CA to select the best calcium carbonate bacterial producers for a wide range of applications.

Figure 1: Graphical abstract.



References

(1) Nigro, L. et al. (2022). Carbonatogenic bacteria on the 'Motya Charioteer' sculpture. Journal of Cultural Heritage, 57, 256-264.
(2) Benedetti F. et al. (2023). Isolation of carbonatogenic bacteria for biorestoration. Journal of Cultural Heritage 64, 282–289.
(3) Lü, X. et al. (2019). Calcium carbonate precipitation mediated by bacterial carbonic anhydrase in a karst cave: Crystal morphology and stable isotopic fractionation. Chemical Geology, 530, 119331.
(4) Supuran, C. T. & Capasso, C. (2017). An overview of the bacterial carbonic anhydrases. Metabolites, 7(4), 56.
(5) Rodriguez-Navarro, C. et al. (2019). The multiple roles of carbonic anhydrase in calcium carbonate mineralisation. CrystEngComm, 21(48), 7407-7423.

PARCA project- Advance in Proteomics and Analysis of dyes and Recovery of Charred and Aged textiles

[1,3]Serafini, I., [2,]***Bosi, A.**, [1]Ciccola, A., [4]Curini, R., [1]Favero, G., [3]Kavich, G. M., [3]Cleland, T. P.

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Archaeological textiles stand as invaluable remnants of ancient civilisations, revered for their profound historical and cultural value. Their delicate state of preservation and the effects of degradation they endure demand the application of highly sensitive analytical tools for comprehensive study. The PARCA project is committed to investigating both components of ancient textiles: dyes, with a specific focus on anthraquinone structures, and keratins (IFs) along with keratin-associated proteins (KAPs). The project aims to develop an innovative protocol to join dye and protein analysis into a single extraction process. This approach not only reduces the number of samples required but also optimises the information obtained from each sample, aimed at a comprehensive approach.
The analysis began with madder-dyed wool mock-ups, and several extraction solutions were tested until the development of two possible methodologies: the TCEP/CAA method (1) or the modified urea method. Following extraction, various clean-up methods, including μ-SPE, stage tips, dLLME (2), or paramagnetic beads (3), were employed to isolate both components, ensuring high analytical sensitivity. Proteins were then digested with trypsin, either in-solution or on beads, and subsequently desalted using C18 SPE/C18 stage tips. Peptides were separated using ThermoScientific Acclaim PepMap 100 trap and analytical columns (300 nL/min, while dyes were separated using a Waters BEH Shield RP18 column with an Ultimate 3000 system (200 uL/min). Both were detected using an Orbitrap Elite mass spectrometer.
The methodology foresaw further testing on laboratory samples subjected to a progressive thermal ramp, ranging from 200°C to 300°C. This simulated the conditions experienced by charred archaeological samples, aiming to determine the survival threshold of proteins and dyes at different thermal aging stages. These findings were correlated with FTIR and SEM data to establish a connection between non-destructive and proteomics analyses. Preliminary results of applying these methodologies to wool samples from the Vesuvian area are also presented.

Acknowledgments
This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101029204. This project was also supported by the Smithsonian Museum Conservation Institute Federal and Trust funds (GMK, TPC).

Funded by the European Union

References

(1) Solazzo, C. & Niepold, T. (2023). A simplified sample preparation for hair and skin proteins towards the application of archaeological fur and leather. Journal of Proteomics, 274, 104821.
(2) Bosi, G. et al. (2023). Molecules, 28, 5290. New Advances in Dye Analyses: In Situ Gel-Supported Liquid Extraction from Paint Layers and Textiles for SERS and HPLC-MS/MS Identification. Molecules, 28,14, 5290.
(3) Cleland, T. P. (2018). Human bone paleoproteomics utilising the single-pot, solid-phase-enhanced sample preparation method to maximise detected proteins and reduce humics. Journal of Proteome Research, 17, 3976–3983.

Microbial communities inhabiting deteriorated frescoes: a metagenomic approach to the case of Santa Maria della Grotta

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In the last years, metagenomic analyses have been exploited to unravel microbial communities colonising cultural heritage objects, buildings and ruins, thus giving new insights on biodeterioration.
In this study, high throughput sequencing of both 16S rRNA gene and 18S rRNA gene amplicons was used to investigate microbial communities settled on frescoes located in two hypogea rooms of the Santa Maria della Grotta Complex in Marsala (Trapani, Italy). Sampling was made in the areas where colour differences were evident and alteration and degradation products were macroscopically identified.
Results indicate a predominance of the Actinomycetia class, the most represented genera being *Pseudonocardia* followed by *Mycobacterium*. In samples where Actinomycetia are less abundant, Gammaproteobacteria are the main colonisers. Interestingly, these results are in agreement with the hypogeal nature of the site. Besides the main represented classes, bacterial communities include, although at low percentages, nitrogen fixators, nitrogen oxidisers, sulphur reducing and chemoorganotrophic and lytotrophic bacteria and Archaea. In sample F1, a 22% of Cloroflexota indicates the occurring of phototrophism. Of note, sample H6 shows a peculiar composition possibly because of the richness in salts of the sampling point. Bacterial communities' composition seems to be driven by the microclimatic conditions of the site and of the nature of the substrates.
As for the 18S amplicons, the class of Saccharomycetes belonging to the Ascomycota phylum, and the Class of Malasseziomycetes belonging to the phylum of Basidiomycota, are the most represented ones. The Embryophyta class is also recorded in some samples, in line with the presence of invasive vegetation in the site. Some soil-typical microfauna and Protists are also present: Chromadorea (Nematozoa), Cercomonadidae (Cercozoa) and Conoidasida (Apicomplexa). An important presence of Arachnida in the sample G3 is also registered.

This work has been partially funded by the European Union (NextGeneration EU), through the MUR-PNRR project SAMOTHRACE (ECS00000022).

Selection and identification of indigenous microorganisms living on spray painted surfaces to be used for biocleaning graffiti

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Millions of heritage and urban surfaces in most cities worldwide are defaced by graffiti vandalism, resulting in economic losses of millions of euros a year. Unfortunately, cleaning protocols based on chemical or physical procedures are not often completely effective. Avant-garde studies have shown that microorganisms offer a powerful, low-cost, safe and eco-friendly solution, to remove graffiti from urban surfaces. However, a clear and fully satisfying biocleaning procedure for spray paints has not yet been achieved.

In this research, a significant contribution to the field was provided by using omics technologies to select promising bio cleaning microorganisms. Graffiti samples were prepared with silver and black spray paints. Adhered bacteria were forced to use paint as the sole source of energy and carbon. At the beginning of the experiment and after 2, 5, 7 and 14 days, the microbial growth was monitored by plate count assay and cellular activity was evaluated by total protein quantification. At three time steps (0, 7, 14 days), the alive biomass was able to grow only in the presence of paints as a nutrient was recovered, the DNA was extracted and the bacterial community was identified by Illumina MiSeqDNA sequencing.

Data revealed that after 14 days, a community of cultivable, alive and active bacterial cells was still present on both silver and black-painted surfaces. Illumina analysis indicated that these communities were mostly composed of two bacteria of the Enterobacteriaceae family.

Microbiological air monitoring and biodeterioration risk in the Cathedral of Santa Maria del Fiore

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The Cathedral of Santa Maria del Fiore in Florence is one of the greatest artistic and architectural masterpieces in the world, and its conservation is a main issue of worldwide concern. Every year, a large number of visitors come from all over the world because of its intrinsic value and for the valuable artworks contained inside. These objects are potentially threatened by microorganisms present inside the Cathedral, which could cause biodeterioration of different materials. A microbiological monitoring campaign of the indoor air and artistic surfaces of the Cathedral has started as part of a collaboration between our Department and the Opera del Duomo. We aim to define the load and composition of the indoor airborne and surface microbiota and to evaluate the biodeterioration risk for the artworks. For these purposes, we are employing culture-dependent methods and are developing an appropriate methodology to obtain a high-quality DNA from air and surfaces for metagenomics. Different areas for air sampling for bacterial and fungal cultivation were chosen: 1) the North and South sides of the Nave (ground floor); 2) near the Altar and close to an air vent system in the Santa Reparata crypt (AV, floor -1); 3) two different levels of the North and South sides of the Dome. To date, we sampled air in two different seasons (end of Summer, and end of Autumn) and have planned further samplings in the 2024 seasons. Preliminary results obtained by cultivation indicated a decrease in the microbial titer (CFU/m^3) from Summer to Autumn in the Central Nave and the opposite trend at the AV site. The microbiological data will be related to microclimatic measurements and tourist flows.

Binder-pigment interaction by proteomic approaches

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The appearance of a painting is the result of the materials used in terms of the pigments present (which determine the color) and the organic binder (which determines the painting technique) (1). However, paintings are not stable or immovable and reactions take place over time, which strongly influence the final look of a painting. Once applied, the paint undergoes chemical reactions resulting in the formation of a dry film. Further chemical changes occur during ageing, such as oxidation and cross-linking of the organic media, reactions between the paint binder and the pigments as well as reactions of the pigments with the atmosphere (1,2). Although modifications arising from binders-pigments interactions may change drastically the appearance and physico-chemical stability of a painting, very little has been published about these changes and research has mainly focused on oily media (1). In particular, very little is known about the nature of the interactions between the inorganic pigments and the proteinaceous binders, and their impact on paint aging under variable environmental conditions (3).

As an initial approach to this problem, we studied the interaction of casein (proteinaceous binder used in the *tempera* technique) with four inorganic pigments that are commonly employed in paintings: azurite ($Cu_3(CO_3)_2(OH)_2$), Saint John's white ($CaCO_3$), cinnabar (HgS) and red lead (Pb_3O_4). Paint reconstructions were prepared by applying pigments mixed with water solutions/dispersions of casein onto glass slides. The resulting paint films had been artificially aged and then scratched to obtain fine powders. The chemical modifications undergone by proteins as an effect of ageing, and depending on the pigment, were investigated by proteomics approaches based on Nano LC-MS/MS (4). Moreover, a LiP-MS-based approach, new to the cultural heritage field, was used to explore how the pigments can influence the 3D structures of proteinaceous binders. This technique enabled the identification of specific protein regions possibly affected by protein binder-pigment interaction (5).

This work was financially supported by PNRR PE5 CHANGES (PE00000020).

References
(1) Duce, C. et al. (2013). Interactions between inorganic pigments and proteinaceous binders in reference paint reconstructions. Dalton Transactions 42, 17, 5975-5984.
(2) Řeháček, K. et al. (1976). Pigment-Binder Interaction in Paints. Industrial & Engineering Chemistry Product Research and Development 15,1, 75-81.
(3) Elert, K. et al. (2018). Pigment-binder interactions in calcium-based tempera paints. Dyes and Pigments 148, 236-248.
(4) Leo G. et al. (2011). Deamidation at asparagine and glutamine as a major modification upon deterioration/aging of proteinaceous binders in mural paintings. Analytical chemistry 83, 62056-2064.
(5) Schopper, Simone, et al. (2017). Measuring protein structural changes on a proteome-wide scale using limited proteolysis-coupled mass spectrometry. Nature protocols 12,11, 2391-2410.

A proposal for a green selective chemical cleaning method of paper artifacts

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In archival-library preservation, degradation phenomena in paper artifacts have mostly been countered by approaches that have not considered the root causes, either due to cognitive limitations or related to existing technologies. The combination of new, highly sensitive diagnostic techniques with the observation of the conduct of reactive molecules in a selective manner may enable the formulation of "preventive conservation" protocols that limit restoration interventions over time. This direction of study could be functional to the evolution of the conservation paradigm in the field of archival-book heritage conservation, as it tends to unite the concept of 'restoration' with that of 'minimal intervention' codified by Cesare Brandi. The research presented here moves in this direction. In light of current knowledge on the phenomenon of "foxing", a type of chemical degradation of paper artefacts, an attempt was made to develop an intervention protocol based on the application of molecules produced in nature by microorganisms and plants that could be used in the future in the containment of chemical agents, acting on a nanoscopic scale, but producing macroscopic effects. The experiment conducted in 2022 by the author of the present study with the support of Professor Laura Micheli, Professor of Analytical Chemistry (Tor Vergata University), was focused on the capture of ferric oxide from oxidised paper artefacts via a siderophore drug that was incorporated in a type of gel that is widely used by paper conservators. A comparative study was conducted between different gel application samples that revealed both the compatibility between the carrier and siderophores and the effectiveness of these molecules in cleaning a paper substrate selectively.

Gamma radiation effects on Cultural Heritage artifacts at Calliope facility (ENEA Casaccia R. C., Rome, Italy)

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Ionising radiation processes are successfully employed in several countries for conservation and preservation treatments of Cultural Heritage (CH) artworks (1). Radiation processing is widely used in CH field for diagnostic and conservation purposes but in Italy the last application is still not usually applied despite the assessed advantages. Therefore, further efforts and experimental tests have to be performed to provide reliable and specific results, obtained by different and commonly applied experimental techniques, to describe the possible secondary-effects occurrence induced by the treatment. Gamma radiation processing is a valid tool for the disinfection and disinfestation of material of cultural interest because of its effectiveness in the inactivation of bio-deteriogen organisms and microorganisms (insects, fungi and molds), often present in damaged artifacts of natural origin (e.g. paper, wood, leather, parchment) and that can represent a risk for the health of CH operators.

In the last years, several activities were performed at the Calliope 60Co gamma irradiation facility (ENEA Casaccia R. C., Rome, Italy) (2) with the aim of investigating the physico-chemical modifications induced by radiation on cellulose-based substrates by means of different experimental techniques, such as Fourier Transform Infrared (FTIR) spectroscopy, Electron Paramagnetic Resonance (EPR) spectroscopy, viscosimetric and colorimetric analysis (3).
The choice of the irradiation parameters (absorbed dose and dose rate) to be used for the treatment is crucial to ensure the biocide efficacy and the minimisation of the secondary-effects, guaranteeing the safeguard of the artifacts.
A case study on an old book, characterised by the presence of fungi and molds (Fig. 1), will be presented. The results, in terms of secondary-effects occurrence, will be compared to those obtained on pure cellulose paper, used as reference material.

Figure 1: Old book attacked by biodeteriogens (a); growth of the microbial community before (b) and after (c) the irradiation treatment.

References

(1) International Atomic Energy Agency, Uses of Ionizing Radiation for Tangible Cultural Heritage Conservation, (2017) IAEA Radiation Technology Series No. 6, IAEA, Vienna.
(2) Baccaro, S. et al. (2019). ENEA Technical Report, RT/2019/4/ENEA.
(3) Cemmi, A. et al. (2022). Gamma radiation-induced effects on paper irradiated at absorbed doses common for cultural heritage preservation, Rad. Phys. Chem., 202.

Advantages in applying long reads metabarcoding in cultural heritage conservation

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Metabarcoding revolutionises ecological analysis by unravelling biodiversity intricacies. While conventional short-read sequencing had limitations in species identification due to fragmentary sequences, the emergence of long-read sequencing addresses these challenges.

Long reads offer advantages such as improved taxonomic resolution, accurate species identification, and enhanced sensitivity for rare taxa. They overcome short-read ambiguities by capturing entire genetic markers like 16S or Internal Transcribed Spaces (ITS), facilitating precise species delineation. Long-read metabarcoding finds applications in environmental monitoring, biodiversity conservation, and microbial ecology, enabling comprehensive community assessments and revealing microbial roles in ecosystems.

Although long-read sequencing has been a reality for many years, its application is very limited due to a lack of software that can utilise long but noisy sequences. New advances in Nanopore sequences reduced the level of sequence errors, allowing for a more accurate identification of genera and species.

In our work, we developed a pipeline to handle long-noisy reads, allowing accurate classification of both 16S and ITS. The pipeline, in combination with an *ad hoc* database, allows the classification of the microbial community at the species level.

Analysis of the fungal communities contaminating museum collections and the fungicidal effects of hydrodistilled plant essential oils: a case study in the Science Museum of the University of Coimbra

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Museum collections are an important part of any culture, helping in the preservation and dissemination of past knowledge, artifacts, and traditions. However, museum collections, alongside other forms of cultural heritage, currently face many threats, including the phenomenon of biodeterioration. With fungal contamination being one of the main causes of cultural heritage biodeterioration, the need to find solutions that can actively prevent and combat it is ever-growing. In the search of these solutions, one must consider their effectiveness as fungicides, their inability to affect aesthetically or structurally the treated artifacts, as well as their environmental impact.
In this study, a polyphasic and multidisciplinary approach is conducted as to characterise the fungal communities present in the Science Museum of the University of Coimbra collection. Afterwards, an analysis of the fungicidal effects of plant essential oils, obtained via hydrodistillation, is performed and their impact on the treated artifacts is assessed. This is crucial to developing a strategy that can be put in place by these and other institutions.
Sampling of artifacts belonging to the Science Museum of the University of Coimbra collection and showing clear signs of fungal contamination was performed, from which several isolates were obtained, belonging to the *Aspergillus*, *Epicoccum*, *Penicillium*, *Talaromyces*, *Trichoderma* and *Wallemia* genera. These isolates were then exposed to varying concentrations of the volatilised fractions of plant essential oils (*Cymbopogon citratus*, *Lavandula angustifolia*, *Margotia gummifera*, *Mentha pulegium*, *Mentha suaveolens* and *Thapsia villosa*), obtained via hydrodistillation, and their impact on fungal growth was assessed. Of the tested oils, most impacted fungal growth, with some being able to fully inhibit the growth of most fungi even at the lowest tested concentrations.

Extraction and characterisation of microalgae-based polysaccharides and protein mixture: potential and innovative material for paper restoration.

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In recent years, there is a strong need to identify sustainable and effective solutions in the conservation field. Microbiology applied to cultural heritage provided an interesting perspective toward sustainable and green solutions. This study aims to produce and physically-chemically characterise a polysaccharide and protein-based mixture, extracted with sustainable methods from microalgae, to be used in the conservation of paper-based artwork.

Figure 1: microalgae and extracts

We have first selected freshwater strains of *Chlamydomonas reinhardtii* (CC125 and SAG 11-32b), marine species of *Porphyridium purpureum sp.* and *Lyngbya sp.*, as sources of endo- and eso-polysaccharides. Hence, the optimal growth conditions for the accumulation of richer polysaccharide-protein mixtures were obtained after the first extractions. Thus, we applied to the culture modulations of temperature, light, and growth medium composition, to maximise the polysaccharide mixture production yield. The microalgae growth was monitored through optical density, cell count, chlorophyll content, and fluorescence efficiency. A green protocol for the extraction of the mixture was developed and optimised, and finally, the extracts were characterised by UV-vis, and FTIR spectroscopies, and by Dynamic Light Scattering to evaluate the average size in solution.

The presence of carbohydrates, nucleic acids, and protein residues, the last two in smaller quantities, was observed. The total amount of carbohydrates and proteins has been determined through the sulfuric acid – UV method, and Bradford assay, respectively. The chemical characterisation has been accomplished by gas chromatography analysis to determine the monosaccharides of the extracted mixtures. These results will provide useful information for future gelation studies, and application tests on paper specimens.

Evaluation of biodeterioration in the UNESCO site Etruscan Necropolis of Tarquinia

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The Etruscan tombs of Tarquinia (Italy), listed as a UNESCO World Heritage Site in 2004, are hypogeal environments characterised by the presence of fine and precious wall paintings. Being carved in rock, these environments host a microbial community that can compromise the conservation of these artworks in specific environmental conditions (1, 3). Indeed, the literature reports how different fungal species, penetrating inside porous stone materials, can cause physical and aesthetic damage leading to the detachment of the paint film or to the production of pigments, respectively. Finally, it is known that some fungal strains can dissolve carbonate substrates as a result of the metabolic production of organic acids (4-6). Aware of these dynamics, in collaboration with the restorer in charge of the archaeological site, the microbial communities within some Etruscan tombs have been monitored for several years. Usually, a degradation phenomenon identified in these environments is the presence of black spots, which are often associated with the presence of black fungi. Through *in vitro* growth and metabarcoding analysis of these spots, several fungal strains were identified, highlighting some similarities with some European Paleolithic caves (7,8). The study of their metabolic activity showed that their presence does not threaten the conservation of artworks from a chemical and/or physical point of view. However, the production by some strains of melanin could affect the aesthetic fruition of mural paintings. These results suggest how a targeted assessment of the metabolic activity of microorganisms can help restorers choose whether and how to proceed with avoiding the biocide treatments. Very often, in fact, such treatments, besides being harmful to the environment and to the operator, are effective on some species, while constituting a carbon source for others, encouraging their proliferation (9). Each microbial community, in fact, is characterised by its own internal balance that must be preserved and maintained.

References

(1) A. Cirigliano et al., Active microbial ecosystem in Iron-Age tombs of the Etruscan civilisation, Environmental Microbiology, vol. 23, 7, pp. 3957–3969, 2021.
(2) A. Cirigliano et al., Calcite moonmilk of microbial origin in the Etruscan Tomba degli Scudi in Tarquinia, Italy, Sci Rep, vol. 8, 1, p. 15839, ott. 2018.
(3) M. C. Tomassetti et al., A role for microbial selection in frescoes' deterioration in Tomba degli Scudi in Tarquinia, Italy, Sci Rep, vol. 7, 1, p. 6027, lug. 2017.

(4) Paiva, D. S. et al. (2022).Uncovering the Fungal Diversity Colonizing Limestone Walls of a Forgotten Monument in the Central Region of Portugal by High-Throughput Sequencing and Culture-Based Methods, Applied Sciences, 12, 20, 10650.
(5) Sterflinger, K. (2010). Fungi: Their role in deterioration of cultural heritage, Fungal Biology Reviews, 24, 1,47–55.
(6) Zucconi, L. et al. (2022).Fungi Affecting Wall Paintings of Historical Value: A Worldwide Meta-Analysis of Their Detected Diversity, Applied Sciences, 12, 6, 2988.
(7) Alonso, L. et al. 2022). Microbiome Analysis of New, Insidious Cave Wall Alterations in the Apse of Lascaux Cave, Microorganisms, 10, 12, 2449.
(8) Sanchez-Moral, S. et al. (2021). Environment-driven control of fungi in subterranean ecosystems: the case of La Garma Cave (northern Spain), Int Microbiol. 24, 4, 573–591.
(9) Villar-dePablo, M. et al. (2023). Innovative approaches to accurately assess the effectiveness of biocide-based treatments to fight biodeterioration of Cultural Heritage monuments, Science of The Total Environment, 897, 165318.

Transcriptome sequencing of xerotolerant fungus *Aspergillus puulaauensis* growing on restoration materials Lascaux acrylic glue 498 and Regalrez 1094

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Artistic paintings on canvas are often subjected to fungal attack when stored under inappropriate conditions. Fungi can cause visible and sometimes structural damage to paintings, mainly due to their ability to grow at low relative humidity and produce various enzymes and organic acids. Paintings can be overgrown by fungi relatively soon after conservation-restoration treatment, even when synthetic restoration materials are used as consolidants and varnishes. Despite the widespread use of synthetic consolidants, little is known about their resilience against fungal growth and the effect of storage conditions.

The poster presents data on the transcriptomic analysis of the xero- and halotolerant fungus *Aspergillus puulaauensis* grown on synthetic materials commonly used in conservation-restoration practices: Lascaux Acrylic Glue 498 (butyl-methacrylate dispersion) and Regalrez 1094 (hydrocarbon resin). The fungus was isolated from several historical paintings and grew abundantly on laboratory mock-ups with synthetic materials applied. Raw sequencing reads were filtered and normalised, and the adapter sequences were removed in fastp. De novo transcriptome assembly was performed in Trinity, a short-read assembly program. The assembled transcriptome was quantified using Salmon. The transcripts were further processed using mmseq2, which facilitates clustering and additional quantification. The completeness or overall quality and integrity of the assembled transcriptome was assessed using BUSCO, which benchmarks the assembly against a set of conserved orthologs. Differential gene expression against the transcriptome of the fungus grown without synthetic material free culture medium was performed using DESeq2 in the R environment, highlighting genes that show significant expression changes under different conditions. Finally, functional annotation of the assembled transcriptome was achieved using Trinotate. The results suggest an activation of cellular stress response pathways alongside the overexpression of enzymes that may be involved in the degradation of the tested restoration materials. Nevertheless, thorough analyses are essential to gain a comprehensive understanding of these results.

In Living Color: exploring the correlation between taxonomic and functional properties of subaerial biofilms and their color

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Stone monuments and buildings can be colonised by microbial communities at the mineral/air interface, known as subaerial biofilms (SABs), which establish complex relations with the environment they inhabit. A common phenotypic trait among all SABs is their pigment-based color, reflecting the physiology of the microbial community. Pigments, associated with several biological functions, represent the link between phenotypic traits, community fitness, and ecological dynamics.
This study aims to investigate the correlation between the SABs' composition and functionality and their specific color profiles. Four distinctively colored SABs—purple, yellow, green, and black—growing on a wall of a chapel located in Milan, were analysed. We studied SABs' gene expression using an innovative metatranscriptomic pipeline, in conjunction with their reflectance spectra. The results showed that the microbial communities of the four colored biofilms and their functional attributes are distinct. The findings from this pilot study will contribute to a deeper understanding of SAB physiology and underscore the potential use of color as a simple and non-destructive monitoring tool for biofilm investigations.

Biodeterioration and Biodegradation of Historic Parks of the UK by Algae

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The present study aims to study the groups of algal genera which are responsible for the biodeterioration, biodegradation and biological pollution of the structures and features of the two historic parks of UK. Different sites of Campbell Park and Great Linford Manor Park in Milton Keynes are selected to study morphological, aesthetic and physical effect of the algal growth.
Specimens and swabs were collected mechanically from selected sites. Algal specimens are preserved in Lugol's solution and labelled with standard information. Photomicrograph analysis of slides using taxonomic keys and visual observation identified algal species that are homogenously and non-homogenously mixed in the aerial, terrestrial and aquatic habitats.
Qualitative study revealed seven classes of Algae. Most of the algal genera isolated have proven records of potential biodegradation, discoloration, and biological pollution. Chlorophyceae was predominantly represented by eleven genera *Chlorella, Chlorococcum Cladophora, Coenochloris Cylindrocapsa. Microspora, Prasiola, Spirogyra, Trentepholia, Ulothrix* and *Zygnema*.
Charophyceae is represented by four genera *Cosmarium Klebsormidium, Mesotaenium* and *Mougeotia*.
Xanthophyceae with two genera *Tribonema* and *Vaucheria*.
Bacillariophyceae (Diatoms) represented by six genera *Acnanthes, Bacillaria, Fragilaria, Gomphonema, Synedra* and *Tabellaria*. Dinophyceae with a Dinoflagellate.
Rhodophyceae included *Bangia* and *Batrachospermum*.
Cyanophyceae with five genera, *Chroococcus, Gloeocapsa, Scytonema, Stigonema* and *Oscillatoria*.

The quantitative analysis by statistical method revealed that Chlorophyceae was the predominant class with eleven genera isolated from different sites of the two parks. *Coenochloris* of Chlorophyceae was isolated from thirteen sites during the study followed by *Gloeocapsa* of Cyanophyceae which is isolated from 12 sites. These two algae impart varying shades of green colour on the surfaces on which they form biofilms.
Prasiola, Vaucheria and Trentepholia were isolated only from Great Linford Park. Trentepholia imparted a significant orange colour to the walls and trees of the sites. The compounds present in algae that are responsible for discoloration are the green pigment chlorophyll, orange pigment β-carotene and yellow pigment quinone.
Mesotaenium, Dinoflagellate, Gomphonema, Fragilaria, *Tabellaria* and 2 unidentified genera were isolated from Campbell Park only.

Largest number of algal genera (25) were isolated from the canal of Campbell Park followed by (21) from the canal at Great Linford Manor Park.
The Algae were found to grow on surfaces of walls, wooden fencings, metal sculptures and railing. The Algae are reported to induce surface erosion, natural weathering and cracking leading to technical and mechanical instability and extensive damage to building materials. The algal biofilms secrete different organic acids which are responsible for bio solubilisation and biodeterioration of the building materials.

The aquatic algal blooms isolated during the study release toxins which are responsible for allergy, skin rashes, vomiting, diarrhoea, fever, muscle spasms, lung and throat infections
The study identifies the places and locations at the historical sites which need to be paid attention. It provides insight into the conservation strategies to overcome the negative impacts of bio-colonisation by algae. Prevention measures by different treatments need to be regularly monitored.

Figure 1: Some Sampling Sites of the Parks and Algal Genera.

Bridging the Gap: Exploring the Relationship Between Biological and Physical Properties of Biofilms on Stone Monuments

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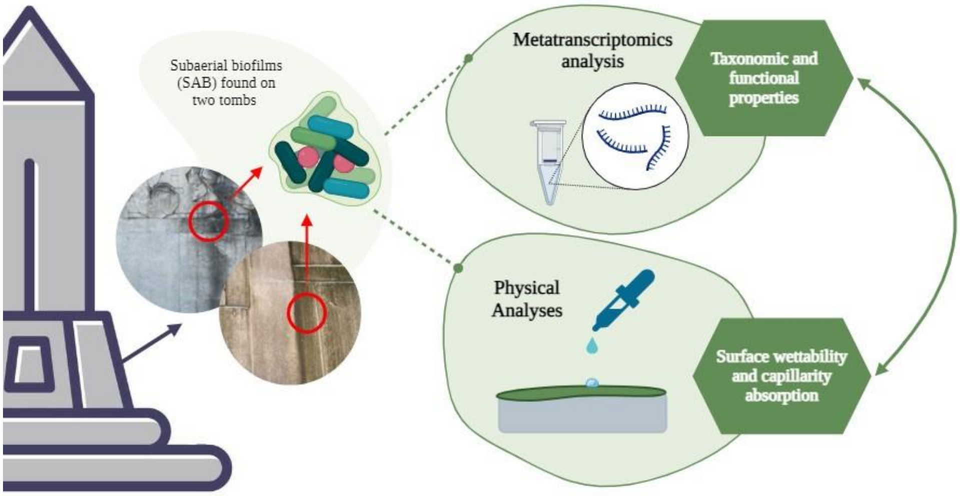

Figure 1. Graphical abstract

Stone monuments, which embody significant historical and artistic testimonies, are colonised by complex microbial communities at solid/air interface, known as subaerial biofilms (SABs). While SABs were previously considered solely as biodeteriogens, recent studies have shown their potential protective role towards the substrate. These researches suggest that SABs regulate the water dynamics between the external environment, the stone, and the biofilm, potentially enhancing the substrate's resilience to deterioration.

The present study aims to investigate the relationship between taxonomic and functional properties of SABs and their effects on water dynamics (Figure 1).

We analysed the SABs found on two tombs located in Milan, each made of two different substrates: Carrara marble and limestone. The SABs were analysed via metatranscriptomic and physical analyses (contact angle and spilling drop test) to explore how the biofilm communities influence water absorption and surface wettability. This study holds promise for enhancing our understanding of SAB ecology and its impact on preservation strategies for stone heritage.

Pink and salty: Exploring the inter-kingdom ecological networks within pink biofilms thriving on salt-weathered lithic substrates

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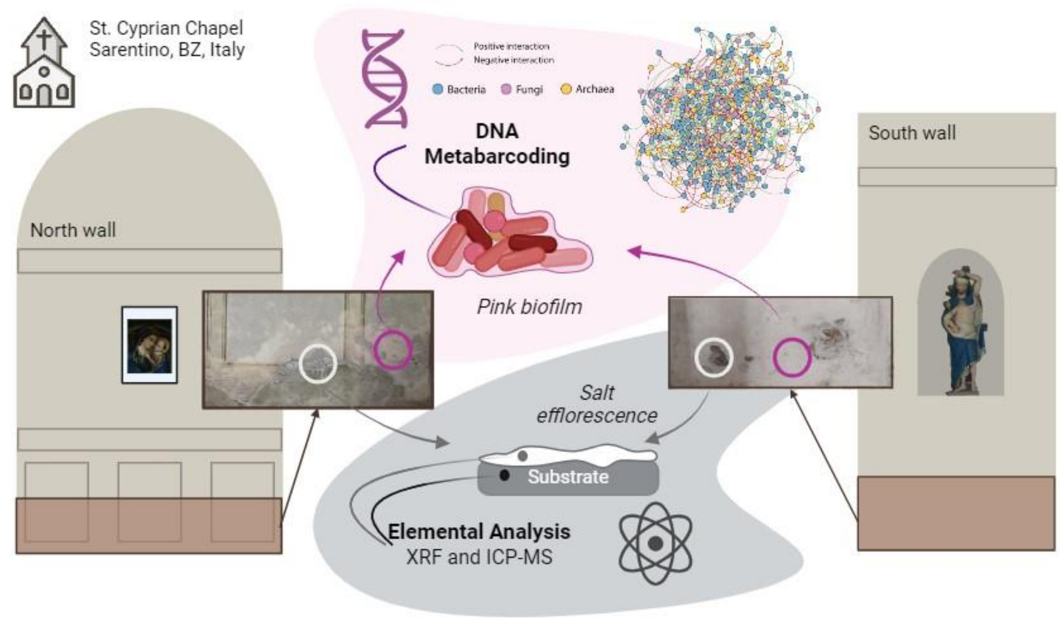

Figure 1. Graphical abstract

Historical buildings face several threats that make their conservation difficult. One significant cause of deterioration is salt weathering, which results from cycles of salt crystallisation and dissolution due to changing environmental conditions. Often associated with this phenomenon is the presence of coloured subaerial biofilms (SABs), which produce pigments that cause a pink colouration. The occurrence of salt efflorescence in combination with the growth of pigmented microorganisms leads to aesthetic alterations and physical and chemical damages.
Here, we investigate the microbial diversity of samples collected in the St. Cyprian Chapel in Sarentino (South Tyrol, Italy) from two walls (north and south) with evident signs of weathering and visible pink patinas. Specifically, we have investigated the intra- and inter-kingdoms'

interaction among archaeal, bacterial, and fungal communities of two pink biofilms growing on two lithic salt-weathered surfaces (Fig. 1).
Furthermore, the salts and the lithic substrates have been characterised by chemical analyses via X-ray fluorescence (XRF), ion-coupled plasma mass spectrometry (ICP-MS) and colourimetric analyses.
Ecological networks play a crucial role in identifying keystone taxa that have important functions in biofilm formation. Understanding these networks can provide valuable insights into the microbiome present on salt-weathered walls and the chemical composition of the salts. These insights can help develop innovative protocols and treatments for the removal of both biofilms and salt efflorescence, thus significantly improving the conservation of historical buildings and monuments.

Terahertz spectroscopy: a complementary approach for Cultural Heritage conservation

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Obtaining insights into the composition of artefacts and finding appropriate strategies to limit deterioration processes and biological contamination of Cultural Heritage materials represent a crucial process for preservation and conservation strategies.
By integrating a multi-analytical approach, conservation scientists can make informed decisions and contribute to safeguarding Cultural Heritage.
In this context, terahertz (THz) spectroscopy can represent a complementary methodology by providing a non-destructive and portable approach. However, there is still a lack of information on the spectral response of substances of interest.
In the field of THz diagnostic for Heritage, implementing the existing datasets and the experimental setups is of crucial importance.
For this reason, specific THz spectra of solid compounds of interest in the Cultural Heritage field are presented.
Furthermore, the identification of Volatile Organic Compounds (VOCs) through THz spectroscopy can envisage a possible selective tracer to detect fungal development and degradation processes.
For this reason, this work reports the characterisation of different VOCs that can be present in historical and archaeological contexts and which can potentially arise from the metabolism of fungi throughout their growth phases or that represent degradation byproducts of organic materials.

Omics at the Musei Reali di Torino. Zooarchaeology by Mass Spectrometry (ZooMS) for taxonomic identification of osseous objects and the detection of restoration glues from legacy collections

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Omics methods provide efficient tools for the study and valorisation of cultural heritage. Among them, paleoproteomic approaches, especially Zooarchaeology by Mass Spectrometry (ZooMS), are increasingly revealing their potential in museum settings. ZooMS is a fast and cost-effective method which has been mainly used to provide science-based identification of raw materials, but can also detect historical restoration treatments on the finds e.g. use of collagen-based glues.

Since 2019 the Musei Reali of Turin has engaged in *'omics* research thanks to a memorandum of understanding (nicknamed *"Bones")* with the ArchaeoBiOmics lab (Department of Life Sciences and System Biology, University of Turin), which included a doctoral research project (Technology Driven Sciences: Technologies for Cultural Heritage, 36th cycle). The research strategy was to select three meaningful case studies of different entities within the Musei Reali legacy collections (a single object, a whole complex of osseous material culture, and a macro case study involving a larger sampling of biological materials from a complex prehistoric site). The aim was to test whether the application of ZooMS could significantly improve the archaeological interpretation of the objects analysed for up-to-date museum valorisation.

Minimally invasive sampling methods (eraser, bag rubbing, removal of < 1mg bone chips) were tested in this study, all giving satisfactory results. The analyses also highlighted interesting aspects of these finds' complex biographies, for example, conservation treatments that had not been recorded or unexpected raw materials (i.e. bovid raw materials in precious Longobard objects which had been classified as entirely made of deer antler). Moreover, the eraser-based method for collagen extraction provided a good tradeoff between invasivity and ease of application. As such, minimally invasive ZooMS analyses will be implemented in future research projects on the organic materials held at the MRT.

Integrated approach to Identify and Counteract Microbial deterioration of Cultural Asset

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Biodeterioration is a complex process that can induce chemical and physical changes, altering the structure of artwork's constitutive materials. New advances in biotechnology and applied microbiology provide information useful for adequate and sustainable conservation strategies. In this study, we propose integrated protocols based on non-invasive/destructive sampling by using nylon (H^+ - positively charged) membrane fragments, followed by microbial cell incubation on Nutrient and/or Sabouraud agar growth media. The grown microbial biomass was then used to obtain genomic and metagenomic DNA from single microbial colonies or from complex microbial biofilms, respectively, depending on microbial loading. To reveal microbial taxa colonising artworks surfaces, the genomic and metagenomic DNAs have been analysed by Polymerase Chain Reaction (PCR)-amplification and sequencing approaches targeting 16S rRNA gene and ITS regions of prokaryotic and eukaryotic microbial cells, respectively. Then, based on these results and in order to define a valid alternative to chemical synthetic biocides, plant bioactive compounds (essential oils), preliminary assayed by Agar Disc Diffusion and Well Plate Diffusion *in vitro* methods, have been utilised to counteract microbial colonisation using artwork surface colonising microbes. Specifically, *Origanum vulgare* L. or *Thymus vulgaris* L. essential oils, of which the chemical composition was determined by GC-MS analysis, have been tested to set up suitable protocols based on green conservation strategy.
This study may contribute to a broader understanding and develop new perspectives on a future generation of biocontrol molecules and methods for setting up environmentally/operator/artwork-friendly strategies.

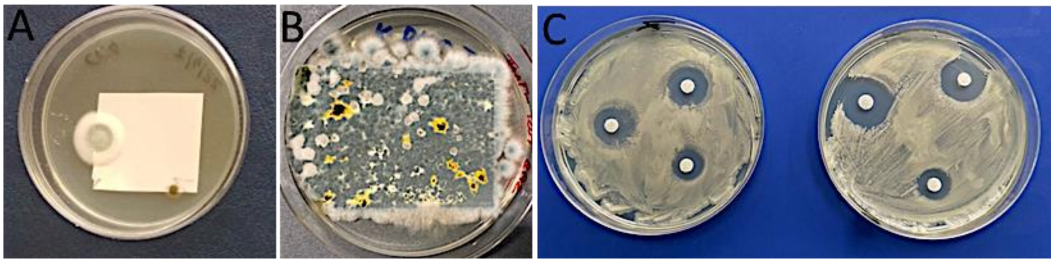

Figure 1: Microbial growth on nylon membrane fragments as single colonies (A) or complex biofilm (B). Antimicrobial activity of *O. vulgare* EO assayed by agar diffusion assay method using selected microbial strains as testers (C).

MinION sequencing: Taxonomical and functional genes identification of the microbiota colonising cultural heritage objects

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The use of Oxford Nanopore Technologies (ONT) sequencing for the analysis of the microbiota responsible for the deterioration of cultural heritage is now a reality. ONT systems, exemplified by the MinION sequencer, offer affordable devices capable of producing long read lengths, providing comprehensive genomic information and reliable identification of microbial species within a community. We describe our expertise in utilising the MinION device to investigate microbiota colonising various cultural heritage samples. Using this ONT sequencer, we successfully sequenced long amplicons of widely used ribosomal genes for taxonomical identification, including 16S rRNA for bacteria and archaea, fungal ITS, and 28S rRNA. Strategies for sequencing functional genes associated with nitrogen cycling (nirK), sulfur cycling (dsr), and sulfur oxidation (soxB) were also developed. Whole genome metagenomic strategies enabled us to investigate microbiota and detect the presence of antibiotic resistance genes, particularly on granite samples previously treated with biocides. Exploiting MinION's capability to sequence long DNA fragments, we sequenced bacterial (around 4000 bp) and fungal (around 3500 bp) ribosomal operons. However, beyond the molecular sequencing aspect lies a significant amount of data processing work, where bioinformatics plays a crucial role. In our bioinformatics workflow, data undergo preprocessing, comprehensive taxonomic classification, and functional annotation steps. We employ state-of-the-art tools to ensure accurate basecalling, adapter trimming, and read filtering. Taxonomic classification of both whole metagenomes and amplicons enhances our understanding of microbiota composition. Additionally, we utilise methods for identifying antibiotic resistance genes, essential for assessing microbial threats to cultural heritage. Our integrated approach, combining advanced bioinformatics techniques, establishes a robust framework for the analysis and interpretation of sequencing data, thus contributing significantly to the preservation of cultural heritage.

Identification of contaminating fungi of taxidermised animals of the Science Museum of the University of Coimbra

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Biodeterioration, defined as any undesirable change in the properties of a material caused by biological agents such as bacteria or fungi, poses significant challenges in the realm of cultural heritage. This process manifests in various cultural artifacts including monuments, wall paintings, stone, wood, paper, and organic works of art like plant and animal fibres and parchment. Understanding the factors contributing to biodeterioration is crucial for effective preservation.

The Science Museum of the University of Coimbra houses collections dating from the eighteenth and nineteenth centuries, spanning anthropology, zoology, mineralogy, and geology. This study aims to identify the fungal species present within items from a bat collection belonging to the Science Museum. Proteolytic activity of obtained isolates was tested using specific culture media, to assess the potential risk posed by identified fungal taxa. It helped distinguishing between harmless colonisation and fungi capable of actively deteriorating the artifacts' support materials.

Samples (obtained through swabbing) were processed and inoculated into Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) media for isolation, and further identification was performed, using molecular and morphological methods. The proteolytic activity was assessed using Skim Milk Agar medium growth tests.

Thirteen bat specimens have been sampled, revealing nearly fifty identified fungal taxa, with prevalent genera being *Cladosporium*, *Penicillium*, and *Talaromyces*. Museums host collections of immense historical and heritage value, which are prone to degradation and even complete destruction by such microorganisms over time. Some of the isolates were shown to have proteolytic activity. Through this research, we seek to unravel the contributing factors to biodeterioration, essential for the prevention and conservation of these invaluable assets. Finally, by keeping these organisms in culture, we will be able to perform different tests towards the development of potential control strategies within the Science Museum.

Essential oils to control microbial growth on the external marble of Florence Cathedral

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The most widely used strategy to contrast biodeterioration of stone cultural heritage so far has been the application of synthetic and aggressive biocides. In recent years, the search for more sustainable strategies in conservation has been developing. Among natural biocides, essential oils (EOs) appear to be highly preferred, due to their well-recognised antimicrobial activity investigated in several fields.

The Cathedral of Santa Maria del Fiore (SMFC) is one of the greatest artistic and architectural masterpieces in the world, and its conservation is a major issue of worldwide concern. The exterior of the Cathedral is mainly covered by Apuan white marble and shows extended forms of decay, including biological patinas.

Here, we report on on-site trials with thyme and oregano EOs as innovative biocides to control microbial growth on the external SMFC white marble. The trials were carried out in two study sites of the Cathedral, differently exposed to alteration, and where marble surfaces showed extended dark discolorations. We first investigated the darkening and composition of the marble microbial community and demonstrated that darkening was mainly due to the growth of black fungi and cyanobacteria. Then we performed preliminary tests with EOs on the SMFC cultivable microbiota and on marble specimens to assess the effectiveness of the treatment and the absence of side-effects on marble. Then, we applied the EOs on selected darkened areas of the two study sites and compared their action with that of Biotin T, a broad-spectrum commercial biocide. The effectiveness of the treatments was assessed through short- and mid-term evaluation by multidisciplinary in situ and ex-situ tests.

A novel mechanism for archeological iron conservation using dead biomass of the yeast *Meyerozyma* sp.

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An environmental strain of the yeast *Meyerozyma* spp. was studied for the removal of chlorides from archaeological iron artifacts. The choice of this strain was made based on its remarkable resistance to extreme conditions such as high salinity and the presence of heavy metals. Preliminary work revealed unique properties of dead biomass of *Meyerozyma* sp. that enable two distinct ways of interacting with corroded iron in an aqueous environment. One of them involves the biosorption of chloride and iron ions onto the cell wall, while the other entails the ability of dead biomass to trigger redox reactions allowing conversion of reactive corrosion products into more stable compounds. The latter was proven via X-ray diffraction (XRD) analysis carried out before and after treatment.
This peculiar behavior of corrosion conversion observed with *Meyerozyma* sp. may be associated with the presence of redox active agents within the dead biomass matrix. Therefore, to shed light on the mechanism driving this transformation, ongoing experiments delve into the dynamics of this electrochemical system and investigate the surface characteristics of the biomass. The use of zeta potential and point of zero charge analyses provided insights into the surface reactivity of the biomass under different pH conditions and allowed an understanding of its potential electrostatic interaction with corroded iron plates. These results, combined with Fourier Transform Infrared spectroscopy (FTIR) analyses of *Meyerozyma* sp. biomass, allowed also to narrow down a number of functional groups present at the cell wall's surface and that were active in the corrosion conversion process. Finally, membrane dialysis was applied to aqueous solutions from suspensions with dead biomass. This was used to size-separate and further characterise present molecules that might be active during the redox reaction process. All these results suggest a novel redox active mechanism that can be used for the conservation of iron objects.

Unveiling microbial communities and material interactions in museum collections: a multi-technique approach using DNA metabarcoding and electron microscopy

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Natural history collections are essential for biodiversity research, and their value in ancient DNA studies is increasing (1). Specimens in these collections are extremely vulnerable to microbial deterioration (2,3), particularly when preserved in enclosures with reduced monitoring.
This study aimed to characterise the microbial communities colonising diverse materials (fur, bone, skin, feathers, horn) in the Civic Zoological Museum of Rome.
Fungal mycelia and bacterial biofilms growing on the specimens (Figure 1) were sampled with adhesive tape and cotton swabs. Total DNA was extracted from the tapes and analysed for quality and concentration. Extracts from different materials were compared. A whole genome amplification was performed to increase the yield of genomic DNA. A scanning electron microscope (ZEISS EVO) equipped with a backscattered electron detector was used to examine samples of different substrates affected by biodeterioration. Variable pressure and high vacuum conditions were employed following specimen fixation and metallisation. Finally, culturable fungi were isolated from materials and identified by micromorphology and molecular barcoding.
Scanning electron microscopy allows for a detailed characterisation of the microbial communities and their interactions with the different organic and mineral materials in museum collections. Deepening our knowledge about this topic is crucial to implementing conservation strategies and protecting these invaluable scientific and cultural resources. This kind of approach is suitable for cultural heritage artefacts as well, as museums often house collections with similar material compositions. Understanding these microbe-material interactions will inform effective preservation strategies, ensuring the longevity of these cultural treasures.
This research is supported by a grant awarded by the International Biodeterioration and Biodegradation Society (IBBS) to Dr. L.Pin.

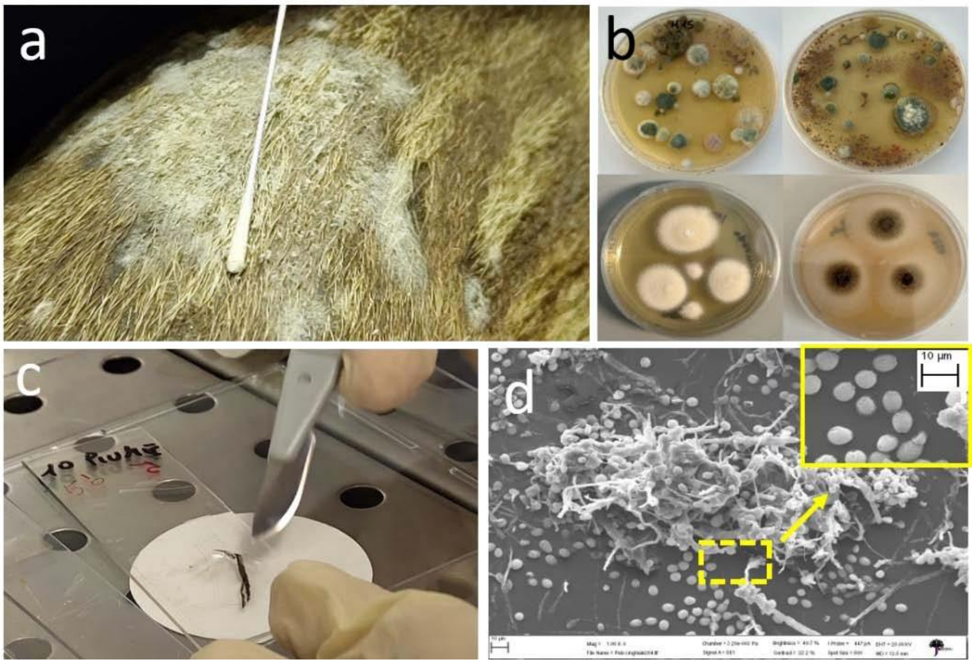

Figure 1: a) swab sampling of mould efflorescence on ungulate skin at the Civic Zoological museum of Rome b) air sampling and isolation of fungal contaminants; c) preparation of glass slides microscopic inspection; d) SEM imaging of moulds' spores and mycelium.

References

(1) Lopez, L. et al. (2020). Genomics of natural history collections for understanding evolution in the wild. Molecular Ecology Resources, 20,5, 1153-1160.
(2) Pinzari, F. et al. (2020). Skeleton bones in museum indoor environments offer niches for fungi and are affected by weathering and deposition of secondary minerals. Environmental microbiology, 22, 1, 59-75.
(3) Planý, M. et al. (2021). Fungal-induced atmospheric iron corrosion in an indoor environment. International Biodeterioration & Biodegradation, 159, 105204.

New conservation techniques based on essential oils encapsulated in an alginate hydrogel system to counteract biofilm growth in the Colosseum Arena

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The study of new green biocides to counteract the presence of biofilms in archaeological sites is a widely discussed topic owing to the increasing number of restorers' requests for alternatives to traditional restoration techniques. Among the different methodologies, Essential Oils (EOs) extracted from plants have emerged as particularly promising biocides due to their well-known content of antimicrobial molecules.
In this work, biofilms growing in the Colosseum Arena (Colosseum Archaeological Park, Rome, Italy) were sampled and maintained in the laboratory to perform tests to assess the biocidal activity of six different EOs extracted from *Ocimum basilicum*, *Cinnamomum verum*, *Lavandula angustifolia*, *Oreganum vulgare*, *Thymus vulgaris* and *Melaleuca alternifolia*. In preliminary screening, the six EOs were applied at 5% concentration on the phototrophic microorganisms isolated from the biofilm and grown on an agarised culture medium to select the biocides with the highest efficacy. Then, the selected biocides were employed against fungi and bacteria isolated from the same biofilm to evaluate the biostatic effects. Moreover, the same EOs at 1% were encapsulated in an alginate hydrogel and applied to phototrophic biofilms grown on Lecce stones.The effect of biocide treatments was assessed by the presence/absence of microorganisms, measurements of photosynthesis activity using the mini-PAM portable fluorometer, observations at optical Microscope (LM), Confocal Laser Scanning Microscope (CLSM) and Scanning Electron Microscopy (SEM).
The data obtained showed that cinnamon EO, but also oregano and thyme were more effective against microorganisms isolated from the Colosseum Arena. The efficacy of these biocides on both phototrophic and heterotrophic microorganisms forming the biofilm, offers the possibility of employing a unique EO-based biocide that could be encapsulated into alginate hydrogel to reduce the required concentration and could also be applied *in situ* where the environmental conditions are complex.

Carotenoid-producing Actinomycetota associated with pink patinas on ancient mural paintings

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Being exposed to both indoor and outdoor conditions, ancient mural paintings are susceptible to microbial colonisation by bacteria, archaea, fungi, and algae, which form biofilms responsible for substrate dissolution and color alterations. The occurrence of the pink patina phenomenon can lead to the deterioration of artwork, and it is likely to be initiated by conditions of elevated salinity and diminished light, which provides favorable environments for the proliferation of pigment-producing halophilic bacteria (e.g., Rubrobacter and Arthrobacter) and Archaea (e.g., Halococcus and Halobacterium).
Our research delves into the pink patina phenomenon observed in two religious structures located in Georgia, namely the Gelati Cathedral (listed on the UNESCO World Heritage List) and the Martvili Monastery.
To characterise the pink patinas on mural paintings within these buildings, we combined physicochemical characterisations with metataxonomic and metagenomic studies, including amplicon-based bacterial biodiversity assessment and the analysis of metagenome-assembled genomes (MAGs). Metagenomic data were integrated with Raman spectroscopy to infer metabolic pathways responsible for pigmentation.

From 10% to 0.1% of phyto-derivatives concentration in five years of application in cultural heritage: how much is the effectiveness of these biocides?

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The development of innovative and sustainable methods to counteract the biodeterioration of cultural heritages due to the growth of microbial communities is a difficult challenge for researchers. In recent years, our research group focused on the employment of phyto-derivatives as "green biocides" that are safer and more eco-friendly than chemicals, also considering the properties of the stone materials that should not be altered. Since 2019, the application of 10% essential oils (Eos) of *Thymus vulgaris* L. and *Lavandula angustifolia* Mill. successfully demonstrated its effectiveness on phototrophic biofilms isolated from Roman Catacombs and did not cause any alteration on painted surfaces (1). Furthermore, considering the phototrophic biofilms growing in the Domus Aurea (Rome, Italy), the effects of liquorice (*Glycyrrhiza glabra* L.) 10% (v:v) leaf extract were evaluated and compared to benzalkonium chloride (0.6% v:v): in this case, the photosynthetic activity of the samples treated with the biocide was significantly reduced (-50%) to 76 days after application (2). Recently, biocides have been encapsulated in alginate hydrogel. This matrix offers the possibility of reducing the oil concentration below 1% and applying it even to vertical surfaces. Promising results have been obtained against cyanobacterial biofilms both in the laboratory and *in-situ* experiments, with a reduction in photosynthetic activity higher than 90%, which remains stable for more than 180 days (3,4). Moreover, five different lithotypes were used for stone colonisation by forming biofilms of biodeteriogens, to evaluate the bioreceptivity of the surfaces and the effects of treatments on them. In this case of study, no alterations of the substrates occurred due to hydrogel encapsulating biocides (5), further demonstrating the possibility of using very low concentrations of Eos (single or mixed) to preserve cultural heritage.

References

(1) Bruno, L. et al. (2019). Biodeterioration of Roman hypogea: The case study of the Catacombs of SS. Marcellino and Pietro (Rome, Italy). Annals of microbiology, 69, 1023-1032.

(2) Rugnini, L. et al. (2020). Biocidal activity of phyto-derivative products used on phototrophic biofilms growing on stone surfaces of the domus aurea in Rome (Italy). Applied Sciences, 10, 18, 6584.
(3) Ranaldi, R. et al. (2022). Plant essential oils suspended into hydrogel: Development of an easy-to-use protocol for the restoration of stone cultural heritage. International Biodeterioration & Biodegradation, 172, 105436.
(4) Bruno, L. et al. (2023). In situ application of alginate hydrogels containing oxidant or natural biocides on Fortunato Depero's mosaic (Rome, Italy). International Biodeterioration & Biodegradation, 183, 105641.
(5) Gabriele, F. et al. (2023). Biodeterioration of stone monuments: Studies on the influence of bioreceptivity on cyanobacterial biofilm growth and on the biocidal efficacy of essential oils in natural hydrogel. Science of The Total Environment, 870, 161901.

Removal of PEG from waterlogged archaeological wood by a bacterial consortium

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To successfully dry waterlogged archaeological wooden artefacts, avoiding severe shrinkages and irreversible deformation, appropriate conservation treatments are required. They have to focus not only on removing water from wood but also on substituting it with materials able to consolidate the degraded wood cell walls (i.e. polymers, sugars or resins).
Impregnation with Polyethylene glycol (PEG) has often been used as the consolidation procedure of choice due to its low cost, stability, and reversibility. However, it has some drawbacks; in particular, it gives corrosion problems in the presence of heavy metals and, due to its hygroscopicity, tends to re-surface, giving the wood a waxy appearance. In some cases, the removal of previous PEG treatments has proven necessary. This procedure requires soaking consolidated wood in a hot water bath, a method that is slow, energy-consuming, and that does not guarantee a complete removal. Hence, there is a need to find a sustainable alternative method.
Biotechnologies based on bacterial metabolism can accelerate the removal process, exploiting the ability of selected bacteria to use PEG as a nutrient source. Two microbial consortia were selected from a consolidating bath containing a mixture of PEG 1500 and 4000 Da, which were able to grow using the two polymers as a 16% (w/v) carbon source. The phylogenetic composition of the two communities was studied using cultural and metagenomic techniques. Both consortia were tested on laboratory consolidated poplar wood samples treated with PEG1500, PEG4000 and a mixture of both. The protocol involved immersion of samples in a mineral medium inoculated with the consortia and incubation at 28°C in an orbital shaker at 100 rpm. PEG removal efficacy was monitored for up to 30 days using optical and electron microscopic techniques. The study found that bacteria enhanced the release of PEG from wood. SEM observations confirmed that bacteria colonised PEG-coated wood.These first results showed that selected bacteria could be effective in speeding up the process of PEG removal in the frame of sustainable restoration practices of waterlogged archaeological wood.

Nanopore sequencing for Biocodicology: a new strategy to study DNA in parchment material

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Biocodicology, a term coined by Sarah Fiddyment in 2019, is an emerging field which studies the biological information stored in *Codex* (parchment manuscripts): the microbiome, fungi and/or bacteria species that colonise the surface of the artefacts and the animal species used for the production of the parchment. The correct identification of these organisms allows us to intervene with a correct restoration/consolidation methodology or can give us more information about the material.
The methods commonly used in this field are culture-dependent or culture-independent methodology to identify the microbiome, and for the animal species, visual evaluation, protein content, or DNA sequencing with Illumina Technology due to the fact that the animal's DNA is usually short and degraded.
This work aims to find an alternative strategy to obtain DNA information from parchment manuscripts through a methodology that combines non-target Whole Genome Amplification (WGA), and DNA sequencing with Oxford Nanopore Technologis.
Several parchments, from the present day to the 15th century, some of them from the Graphic Collection of the Academy of Fine Arts, Vienna, were analysed. Small pieces of the parchments were subjected to DNA extraction followed by Whole Genome Amplification (WGA) and sequencing with the device MinION (MK1C) from Oxford Nanopore Technologies using the new Flow Cells (R.10) and the new Short Fragment Mode (SFM).
The bioinformatic results show that the parchment microbiomes are composed of typical human skin flora, with bacteria belonging to species of the genera *Acinetobacter*, *Moraxella* and *Staphylococcus* as well as fungi (i.e. genus *Malassezia*). More importantly, results enable the identification of the animal species used for the parchment in any analysed case, showing for the first time the potential of WGA and Nanopore sequencing in the field of biocodicology for the identification not just of fungi and bacteria but also for short degraded DNA in parchment.

Fungal microbiome on deteriorated historic paintings

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Fungi are cosmopolitan microorganisms known for their role in biodeterioration. Easel paintings are particularly vulnerable because they contain many organic components that are useful to fungi. Fungi colonise surfaces through air and dust deposits, developing colonies on the *recto* and *verso* sides of paintings and causing deterioration. The most important characteristic for their successful colonisation and growth is xerophily, which allows them to be active at low water activity, even under the recommended microclimatic conditions (50-60% RH). Understanding and controlling the growth of fungi on paintings requires multidisciplinary approaches and the use of state-of-the-art molecular techniques combined with classical techniques such as microscopy, the application of cultivation techniques and *in vitro* laboratory tests. The microbiomes (fungi, bacteria) of six historical biodeteriorated paintings from churches and museums were analysed using Illumina amplicon sequencing of targeted, PCR-amplified genes (ITS2 for fungi, partial 16S rRNA for bacteria) allowing community identification. The total microbiome versus the living microbiome was detected using a DNA modification stain, propidium monoazide, prior to the isolation of the total DNA. In parallel, quantitative PCR of total and active microbiomes allowed us to determine the active portion of fungi and bacteria. Due to the non-specificity of the amplified target genes the use of culturable methods was crucial for species identification according to golden standards (DNA sequencing of ITS or housekeeping genes). It also allowed *in vitro* testing of the key species in pure cultures, as well as sequencing of their genomes and transcriptomes (see Kujović et al. poster). The majority of the paintings studied showed old, inactive contamination, while in some recently restored paintings a high proportion of active microbiome confirmed recent post-restoration contamination. Although xerophilic *Aspergillus* species are the most commonly encountered molds on paintings, there are evidences of some unculturable taxa as well.

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